呼吸疾病国家重点实验室年报

2011 年

SKLRD 2011

呼吸疾病国家重点实验室

2011 年年度报告

前言

2011 年，重点实验室承接上一年度的建设成果，继续夯实基础，探索创新管理，积极引进人才，加快拓展平台。

重点实验室在省市政府、学院和医院领导的支持下，本年度各项科研工作进展顺利，并取得了佳绩。首先，实验室充分利用建设经费投入到仪器购置、实验耗材、平台环境改造、人才引进及培养等方面，经费总体使用情况基本符合最优配置的原则。实验室在科研方面保持优势，在国自然、省自然、青年基金等都获得了多项科研项目，高质量论文发表的数目稳步增长。其次，实验室在年初制订了《呼吸疾病国家重点实验室管理规章制度》、《国家重点实验室外聘 PI（课题负责人）制度》等重要文件，这标志着实验室建设全面步入有章可循的规范阶段，同时，这也成为实验室成功通过卫生部评估的关键因素。实验室这一重大举措旨在推进规范化、标准化的深入贯彻，为探索创新管理找一条新路子，形成更加科学的、更具有活力的体制机制。再次，在人才培养方面，实验室以环境创新和管理创新打造吸引高层次人才的新优势，吸引国内外的精尖人才在实验室“安家置宅”；同时注重以载体创新和制度创新搭建培养内部骨干人才的新平台，实验室设立了青年基金，对入选的优秀项目进行资助，双管齐下的人才计划以期为实验室拓展平台奠定了智力支撑。

今年，实验室凭借着在抗击非典、防治甲流中卓著的贡献，以及在长期发展中以自主创新为核心的机制，荣获首届南粤功勋（团队）

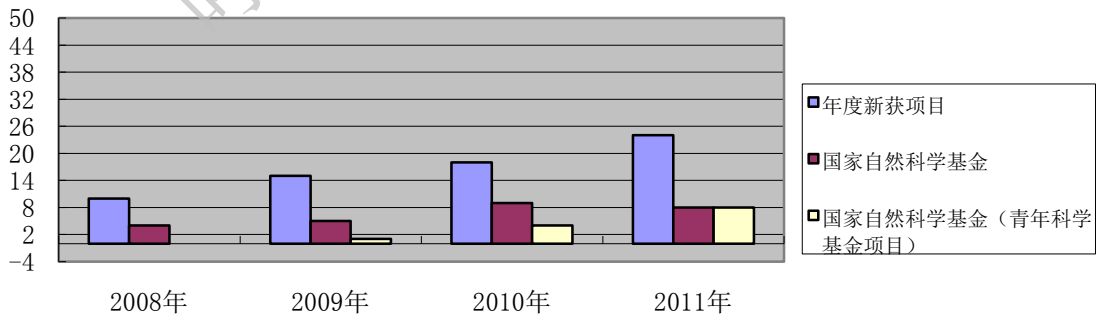
奖，并获得 3000 万的建设经费，这是省市政府对重点实验室在呼吸学领域所做贡献的一种肯定，这也将成为实验室致力创新的强劲动力。此外，实验室在继去年成功申报广东省工程技术研究开发中心后，又积极申报了教育部工程技术研究中心，现已进入审核阶段。本年度，实验室还圆满完成了 973 项目中期汇报，并就项目成果进一步调整部署。

今年，实验室仍为打造具有国家中心城市标志性的广州呼吸中心积极筹备工作，与此同时，继续大力扶持具有自主创新核心能力的产学研平台，在华南新药创制中心成立实验室。展望“十二五”计划，实验室将以科研工作为抓手促进技术创新、增强自主创新，在“十二五”开局之年，深化体制改革，推进实验室各项工作稳步前进，迈上新台阶。

第一部分：实验室科研项目及成果

一、 科研项目和科研经费

本年度，实验室新获项目共 24 项，科研基金累计 5418.1 元，实到金额 1390.75 万元。其中国家自然基金 24 项，其中面上项目 8 项，青年科学基金 6 项，973 项目子课题 2 项，中科院重要方向性项目 1 项，中国科学院创新工程重要研究方向项目 1 项，广东省科技厅产学研项目 1 项，中国科学院支撑服务国家战略性新兴产业科技行动计划专项项目 1 项，省级课题(高等学校博士学科点专项科研基金博导类课题)2 项，中国科学院产业化项目(佛山产业中心扶持资金)1 项，广东省科技厅 1 项。据统计显示，实验室新获项目的数量逐年上升，国家自然科学基金项目持续增长。值得可喜可贺的是，今年，在实验室大力培养青年骨干人才的政策下，青年科学基金项目获得数目有显著的提高。在未来几年里，实验室将继续紧紧围绕突发性呼吸系统传染病、哮喘与慢性咳嗽、慢性阻塞性肺炎（COPD）和肺癌四大方向，积极组织申报各类项目，重点培养青年骨干的科研能力，做到既重量又重质，进一步提升实验室整体的科研能力。



2008 年-2011 年各类新获项目对比表

2011 年主要新获基金项目一览表

序号	项目名称	项目性质	项目批准号	总经费(万元)	负责人	开始时间	结束时间
1	lincRNA 在苯并(a)芘诱发肺癌变中的作用	国家自然科学基金-面上项目	81370177	70.00	蒋义国	2011 年 1 月	2015 年 12 月
2	lincRNA 在苯并(a)芘诱发肺癌变中的作用	国家自然科学基金-面上项目	81470233	60.00	冉丕鑫	2011 年 1 月	2015 年 12 月
3	上皮细胞转分化在生物燃料烟雾致慢性阻塞性肺疾病气道重塑中的作用研究。	国家自然科学基金-面上项目		50.00	刘劲松	2011 年 1 月	2015 年 12 月
4	友菌素糖基转移酶 AmiGT 的结构生物学研究	国家自然科学基金-面上项目	81373176	45.00	王健	2011 年 1 月	2015 年 12 月
5	丹参酮 IIA 在 PAH 治疗中的降钙机制研究	国家自然科学基金-面上项目	81470246	23.00	杨巧媛（蒋义国组）	2011 年 1 月	2014 年 12 月
6	环境致癌物诱发 miRNA 基因突变位点的寻找及其对肺癌发生的影响	国家自然科学基金-面上项目	81400018	23.00	张岚(吕嘉春组)	2011 年 1 月	2014 年 12 月
7	自噬途径在人支气管上皮细胞恶性转化中的作用及其机制研究	国家自然科学基金-面上项目	81400073	22.00	江庆萍(吕嘉春组)	2011 年 1 月	2014 年 12 月
8	EB 病毒 microRNA 调控人 MAK3K5 基因在鼻咽癌发生发展中的作用	国家自然科学基金-面上项目	81403167	21.00	朱春燕(吕嘉春组)	2011 年 1 月	2014 年 12 月
9	妊娠期代谢性疾病孕妇产后代谢综合征的代谢组学研究	国家自然科学基金-面上项目	81470234	23.00	谢佳星	2011 年 1 月	2014 年 12 月

序号	项目名称	项目性质	项目批准号	总经费(万元)	负责人	开始时间	结束时间
10	TRPV1 和 TRPA1 在变应性鼻炎继发下气道炎症及高反应性中的作用	国家自然科学基金-青年科学基金	81573575	23.00	陈莉延（赖克方组）	2011 年 1 月	2014 年 12 月
11	小鼠咳嗽检测方法的建立和小鼠咳嗽自动检测软件的改良	国家自然科学基金-青年科学基金	81570092	14.00	邓方阁	2011 年 1 月	2012 年 12 月
12	合并静脉炎的深静脉栓塞红外辐射轨迹特征的研究	国家自然科学基金-青年科学基金	91542104	14.00	肖大凯	2011 年 1 月	2012 年 12 月
13	上皮间质转化在非小细胞癌吉西他滨耐药形成中的作用	国家自然科学基金-青年科学基金	81471937	23.00	巨春蓉	2011 年 1 月	2014 年 12 月
14	肌抑制素与慢性阻塞性肺疾病骨骼肌无力的关系及其临床意义	国家自然科学基金-青年科学基金	81473040	23.00	秦茵茵	2011 年 1 月	2014 年 12 月
15	COPD 患者呼吸困难缓解机制中呼吸中枢驱动和细胞因子的变化	国家自然科学基金-青年科学基金	2014AA093514	50.00	蒋义国	2011 年 1 月	2016 年 12 月
16	环境铅暴露致儿童脑发育损伤的机制研究	973 项目子课题	2014ZX09102044-006	129.25	刘劲松	2011 年 1 月	2016 年 12 月
17	蛋白质分拣与转运以及降解的结构生物学研究	973 项目子课题	2015ZX09J15105-002	37.00	张天宇	2011 年 1 月	2013 年 12 月
18	重大传染病的疫苗和药物研发关键技术研究	中科院重要方向性项目	KSCX2-EW-J27	40.00	陈小平	2011 年 1 月	2013 年 12 月
19	重大疾病防治的关键技术及药物研究	中国科学院创新工程重要研究方向项目	2011B090400589	30.00	何建行	2011 年 1 月	2013 年 3 月
20	手术切口保护器的应用、产业化及推广	广东省科技厅产		80.00	彭涛	2011 年 1 月	2014 年 12 月

序号	项目名称	项目性质	项目批准号	总经费(万元)	负责人	开始时间	结束时间
		学研项目					
21	用于突发、新发传染病病原现场快速排查的核酸诊断系统的研发与推广	中国科学院支撑服务国家战略性新兴产业科技行动计划专项项目	20114423110002	12.00	蒋义国	2011 年 1 月	2014 年 12 月
22	循环 miRNA 作为环境致癌标志物的实验和人群研究	省级课题(高等学校博士学科点专项科研基金博导类课题)	20114423110002	12.00	蒋义国	2011 年 1 月	2014 年 12 月
23	循环 miRNA 作为环境致癌标志物的实验和人群研究	省级课题(高等学校博士学科点专项科研基金博导类课题)	ZNGI-2011-009	60.00	张健存	2011 年 1 月	2013 年 12 月
24	新型靶向抗肺癌药物 Z582 的临床研究及产业化	中国科学院产业化项目(佛山产业中心扶持资金)	2011B061200040	5.00	孙宝清	2011 年 1 月	2014 年 12 月

2011 年主要在研课题一览表

序号	项目名称	项目性质	项目批准号	总经费(万元)	负责人	进度	开始时间	结束时间
1	骨形成蛋白 4 在缺氧性肺动脉高压中的作用	国家自然科学基金-面上项目	81500017	36.00	王健	在研	2010 年 1 月	2013 年 12 月
2	嗜酸细胞性支气管炎—变应性鼻炎发展为支气管哮喘的中间环节?	国家自然科学基金-面上项目	81572037	33.00	赖克方	在研	2010 年 1 月	2013 年 12 月
3	人 3 型腺病毒-肠道病毒 71 型主要中和抗原表位嵌合体构建及应用基础研究	国家自然科学基金-面上项目	81500046	32.00	周荣	在研	2010 年 1 月	2013 年 12 月
4	蛋白磷酸酶 PPM1E 与 NF-κB/p65 的相互作用及其作为肿瘤治疗新靶标的研究	国家自然科学基金-面上项目	31570759	18.00	王国新（张必良组）	在研	2010 年 1 月	2013 年 12 月
5	WWOX 基因拷贝数和单核苷酸遗传变异影响人群肺癌发病的分子流行病学研究	国家自然科学基金-面上项目	31570759	10.00	吕嘉春	在研	2010 年 1 月	2011 年 12 月
6	肺静脉平滑肌在低氧性肺动脉高压发病中的作用机制	国家自然科学基金-青年科学基金	81471937	18.00	彭公永	在研	2010 年 1 月	2014 年 12 月
7	咳嗽反射可塑性改变在上气道咳嗽综合征发病中的作用及机制	国家自然科学基金-青年科学基金	81471989	19.00	陈如冲	在研	2010 年 1 月	2013 年 12 月
8	转基因表达肿瘤相关抗原 MUC-1 的减毒疟原虫感染治疗小鼠 Lewis 肺癌的实验研究	国家自然科学基金-青年科学基金	81570028	20.00	何正祥（陈小平组）	在研	2010 年 1 月	2013 年 12 月

序号	项目名称	项目性质	项目批准号	总经费(万元)	负责人	进度	开始时间	结束时间
9	慢性呼吸疾病的预防与规范诊治体系建设及适宜技术研究	卫生公益性行业科研专项	2014M562156	260.00	冉丕鑫、钟南山、赖克方	在研	2010 年 1 月	2014 年 12 月
10	丹参酮ⅡA 治疗肺动脉高压的机理研究	省教育厅科技-创新重点项目	cxzd1025	30.00	王健	在研	2010 年 1 月	2013 年 12 月
11	骨形成蛋白 4 增强大鼠远端肺动脉平滑肌细胞内钙池操纵性钙内流的机制研究	广东省高层次人才项目		60.00	王健	在研	2010 年 1 月	2013 年 12 月
12	用于传染病病原现场快速检测的核酸诊断系统的研发	广东省科技计划项目	2011B060100004	40.00	彭涛	在研	2010 年 1 月	2013 年 12 月
13	人 3 型腺病毒六邻体表面抗原的鉴定及特性研究	省自然科学基金-青年项目	10451018201005825	3.00	李潇	在研	2010 年 1 月	2012 年 12 月
14	人 3 型腺病毒中和抗原表位及其应用基础研究	省自然科学基金	10151009504000006	5.00	周荣	在研	2010 年 1 月	2013 年 12 月
15	基于膈肌肌电的自适应呼吸机研制与开发	广东省省部产学研引导项目	2010B090400470	20.00	郑则广	在研	2010 年 1 月	2012 年 12 月

二、 2011 年发表的 SCI 文章一览表

2011 年共发表 SCI 文章 144 篇，其中影响因子大于或等于 20 的有 1 篇，大于或等于 10 的有 3 篇，大于或等于 5 的有 13 篇，大于或等于 3 的有 39 篇，平均影响因子为 3.4898 。

序号	题名	作者	PI 通讯作者	期刊名称	出版年	卷期页	影响因子
1	COULD LEUKOTRIENE D4 BRONCHIAL PROVOCATION TEST BE A CLEAR INDICATOR FOR PREDICTING THERAPEUTIC OUTCOMES OF LEUKOTRIENE RECEPTOR ANTAGONIST? A PILOT STUDY	Guan, W.; Gao, Y.; Jiang, C.; An, J.; Yu, X.; Liu, W.; Zheng, J.	Jingping Zheng	中华医学会呼吸病学年会	2011	16,211-211	2.856
2	FACTORS ASSOCIATED WITH ALLERGEN SENSITIZATIONS IN PATIENTS WITH ASTHMA AND/OR RHINITIS IN CHINA: A MULTICENTRE EPIDEMIOLOGICAL STUDY	Li, J.; Huang, Y.; Lin, X.; Zhao, D.; Tan, G.; Wu, J.; Zhao, H.; Zhao, J.; Spangfort, M. D.; Lai, X.; Zhong, N.	Zhong，N	American Journal of Rhinology & Allergy	2011	16,216-216	2.856
3	Translational medicine: What is in a name from the perspective of Chinese clinicians?	Zeng GuangQiao; Zhong NanShan	Zhong，NS	中国科学:生命科学	2011	54(12),1077-1080	1.772
4	PRESENCE OF BACTERIA FROM ASTHMATIC AIRWAYS: RELATIONSHIP TO SEVERITY	Zhang, Q.; Liang, Z.; Gibeon, D.; Hui, Christopher K.; Alshafi, Al K.; Menzies-Gow, A.; Bhavsar, Pankaj K.; Duff, R.; Moffatt, M.; Cookson, B.; Chung, K. F.	Zhang, Q	RESPIROLOGY	2011	16,220-221	2.856
5	Compliance with the CURB-65 score and the consequences of non-implementation	Guo, Q.; Li, H-Y.; Zhou, Y-P.; Li, M.; Chen, X-K.; Liu, H.; Peng, H-L.; Yu, H-Q.; Chen, X.; Liu, N.; Liang, L-H.;	Jiang, M	International Journal	2011	15(12),1697-1701	2.161

		Zhao, Q-Z.; Jiang, M.		of Tuberculosis & Lung Disease the Official Journal of the International Union Against Tuberculosis & Lung Disease			
6	IS BRONCHIAL PROVOCATION TEST INDUCED BY ADENOSINE MONOPHOSPHATE (AMP) SUPERIOR TO HISTAMINE FOR IDENTIFYING PATIENTS WITH ASTHMA?	Wu, F.; Gao, Y.; An, J-Y; Xie, Y-Q; Liu, W-T; Yu, X-X; Zheng, J-P	Zheng, J-P	RESPIROLOGY	2011	16,221-221	2.856
7	Prognostic Impact of MMP-2 and MMP-9 Expression in Pathologic Stage IA Non-Small Cell Lung Cancer	Shao, Wenlong; Wang, Wei; Xiong, Xin-Guo; Cao, Christopher; Yan, Tristan D.; Chen, Guoqin; Chen, Hanzhang; Yin, Weiqiang; Liu, Jun; Gu, Yingying; Mo, Mingcong; He, Jianxing	He, Jianxing	Journal of Surgical Oncology	2011	104(7),841-846	2.888
8	The expression of p33(ING1), p53, and autophagy-related gene Beclin1 in patients with non-small cell lung cancer	Liu, Jun; Lin, Yongping; Yang, Haihong; Deng, Qiuhua; Chen, Guoqin; He, Jianxing	He, Jianxing	《Tumor Biology》	2011	32(6),1113-1121	2.904

9	DIFFERENT COURSES WITH INHALED CORTICOSTEROIDS FOR EOSINOPHILIC BRONCHITIS	Xu, D-Y; Lai, K-F; Xie, J-X; Chen, R-C; Lou, W.; Zhong, N-S	Zhong, N-S	European Respiratory Journal	2011	16,221-222	2.856
10	A non-synonymous polymorphism Thr115Met in the EpCAM gene is associated with an increased risk of breast cancer in Chinese population	Jiang, Lan; Zhang, Chun; Li, Yinyan; Yu, Xiao; Zheng, Jian; Zou, Ping; Li, Yuting; Bin, Xiaonong; Lu, Jiachun; Zhou, Yifeng	Lu, Jiachun	Breast Cancer Research and Treatment	2011	126(2),487-495	4.145
11	THE PROGNOSIS AND CLINICAL FEATURE OF EOSINOPHILIC BRONCHITIS	Xu, D-Y; Lai, K-F; Lin, L.; Chen, R-C; Xie, J-X; Lou, W.; Zhong, N-S	Zhong, N-S	RESPIROLOGY	2011	16,221-221	2.856
12	A COMPARISON OF PREVALENCE OF ASTHMA AND ALLERGIC RHINITIS BETWEEN THE URBAN AND RURAL ADOLESCENTS IN GUANGZHOU	Chen, Y.; Li, J.; Zhong, N.	Li, J	RESPIROLOGY	2011	16,223-223	2.856
13	Complete genome analysis of a novel E3-partial-deleted human adenovirus type 7 strain isolated in Southern China	Su, Xiaobo; Tian, Xingui; Zhang, Qiwei; Li, Haitao; Li, Xiao; Sheng, Huiying; Wang, Youshao; Wu, Houbo; Zhou, Rong	Zhou, Rong	Virology Journal	2011	8	2.249
14	INFLUENCE OF HISTAMINE NASAL CHALLENGE ON LOWER AIRWAY REACTIVITY	Xie, Y.; Zheng, J.; Chen, Q.; Lai, K.; Qin, Y.; Liang, J.; Hu, Y.	Zheng, J	RESPIROLOGY	2011	16,227-227	2.856
15	A Practical Synthesis of 2-Aroylindoles from N-(2-Formylphenyl)trifluoroacetamides in PEG-400	Zhao, Yu; Li, Deyao; Zhao, Liwen; Zhang, Jiancun	Zhang, Jiancun	Synthesis	2011	(6),873-880	2.348
16	PREVALENCE OF ASTHMA AND RHINITIS AND THEIR TRENDS AMONG ADOLESCENTS IN GUANGZHOU CITY OVER THE PAST 15 YEARS	Chen, Y.; Li, J.; Zhong, N.	Zhong，N	Zhonghua Yi Xue Za Zhi	2011	16,228-228	2.856
17	Establishment of airway eosinophilic bronchitis mouse	Chen, Liyan; Lai, Kefang; Xie,	Lai, KF	Clinical	2011	11(1),19	2.442

	model without hyperresponsiveness by ovalbumin	Jiaxing; Zhong, Nanshan		and Experimental Medicine		-24	
18	EFFECT OF OBSTRUCTIVE SLEEP APNEA-HYPONEA SYNDROME ON ACUTE MYOCARDIAL INFARCTION	Zhang, W.; Sun, Y.; Li, T-D; Zhang, G-M; Wang, Y.; Sun, H.	Wang Y	Bratislavske Lekarske Listy	2011	16,244-244	2.856
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20	HEMAGGLUTININ OF H5N1 VIRUS ACTIVATES JANUS KINASE 3 TO DYSREGULATE INNATE IMMUNITY	Chen, M.; Xu, W.; Ge, N.; Xu, J.	Jun Xu	Plos One	2011	16,265-266	2.856
21	NONINVASIVE PULMONARY FUNCTION ANALYSIS IN THE MURIN PULMONARY FIBROSIS INDUCED BY BLEOMYCIN	Sun, H.; Tan, Y.; Liu, R.; Zhu, Q.; Zeng, Q.; Xiao, D.	Zhu, Q	RESPIROLOGY	2011	16,290-291	2.856
22	Golgi phosphoprotein 2 (GOLPH2/GP73/GOLM1) interacts with secretory clusterin	Zhou, Yan; Li, Leike; Hu, Longbo; Peng, Tao	Peng Tao	Molecular Biology Reports	2011	38(3),1457-1462	1.777
23	The expression and clinical significance of CLIC1 and HSP27 in lung adenocarcinoma	Wang, Wei; Xu, Xin; Wang, Wujun; Shao, Wenlong; Li, Liping; Yin, Weiqiang; Xiu, Liangchang; Mo, Mingcong; Zhao, Jin; He, Qingyu; He, Jianxing	He, Jianxing	Tumor Biology	2011	32(6),1199-1208	2.904
24	Synergy of the antiretroviral protease inhibitor indinavir and chloroquine against malaria parasites in vitro and in	Li, Xiaofen; He, Zhengxiang; Chen, Lili; Li, Yayong; Li, Qinyan; Zhao,	Chen, XP	Parasitology Research	2011	109(6),1519-152	2.096

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25	Effect of plasmid-mediated RNA interference targeting telomerase reverse transcriptase on lung cancer cells	Ge, Linhu; Deng, Zhansheng; Zhang, Yangde; Shao, Wenlong; Qiu, Yuan; Cui, Dong; Huang, Donghai	Donghai Huang	Oncology Reports	2011	26(6),1487-1495	2.488
26	The Golgi Localization of GOLPH2 (GP73/GOLM1) Is Determined by the Transmembrane and Cytoplamic Sequences	Hu, Longbo; Li, Leike; Xie, Hongbin; Gu, Yanli; Peng, Tao	Peng Tao	Plos One	2011	6(11)	3.535
27	Automated Analysis of Time-Lapse Imaging of Nuclear Translocation by Retrospective Strategy and Its Application to STAT1 in HeLa Cells	Han, Fujun; Liang, Peizhou; Wang, Feifei; Zeng, Lingyun; Zhang, Biliang	Zhang, Biliang	Plos One	2011	6(11)	3.535
28	AIDINAFIL INHIBITS CHRONICALLY HYPOXIC UPREGULATION OF CANONICAL TRANSIENT RECEPTOR POTENTIAL EXPRESSION IN RAT PULMONARY ARTERIAL AND VENOUS SMOOTH MUSCLE	Chen, X.; Ran, P.; Ou, H.; Zhong, N.; Wang, J.	Wang, J	American Journal of Physiology Cell Physiology	2011	16,297-299	2.856
29	DEVELOPMENT OF AN EFFICIENT AND SPECIFIC SIRNA-DELIVERY VECTOR FOR RNAI THERAPY IN PULMONARY DISEASES	Luo, Y.	Luo, Y	RESPIROLOGY	2011	16,10-11	2.856
30	IDENTIFICATION OF THE LIMITED CAPACITY OF HADV-3 HVR1	Zhong, T.	Zhong, T	RESPIROLOGY	2011	16,27-27	2.856
31	THE IRTI AND CPUS DOUBLE-BLIND CONTROLLED CLINICAL STUDY ON DVT	Deng, F.; Zhong, N.; Zheng, Y.; Zhang, N.; Tang, Q.; Deng, X.	Zhong, N	RESPIROLOGY	2011	16,300-300	2.856
32	Screening for sleep-disordered breathing in neuromuscular disease using a questionnaire for symptoms associated with diaphragm paralysis	Steier, J.; Jolley, C. J.; Seymour, J.; Teschler, H.; Luo, Y. M.; Polkey, M. I.; Moxham, J.	J. Steier	European Respiratory Journal	2011	37(2),400-405	7.284

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34	High prevalence of plasmid-mediated quinolone resistance determinant aac(6 ')-Ib-cr amongst Salmonella enterica serotype Typhimurium isolates from hospitalised paediatric patients with diarrhoea in China	Yu, Fangyou; Chen, Qiang; Yu, Xiaojun; Pan, Jingye; Li, Qiaoqiao; Yang, Lehe; Chen, Cong; Zhuo, Chao; Li, Xiaoqiang; Zhang, Xueqing; Huang, Jinwei; Wang, Liangxing	Wang, Liangxing	International Journal of Antimicrobial Agents	2011	37(2),152-155	4.349
35	Crystallization and preliminary crystallographic studies of a cysteine protease inhibitor from the human nematode parasite Ascaris lumbricoides	Liu, Sanling; Dong, Jianmei; Mei, Guoqiang; Liu, Guiyun; Xu, Wei; Su, Zhong; Liu, Jinsong	Liu, JS	Acta Crystallographica Section F	2011	67,228-230	0.502
36	Indoor allergen levels in Guangzhou city, southern China	Zhang, C.; Gjesing, B.; Lai, X.; Li, J.; Spangfort, M. D.; Zhong, N.	Zhong，N	Allergy	2011	66(2),186-191	5.746
37	Subcellular proteomics revealed the epithelial-mesenchymal transition phenotype in lung cancer	Li, Li-Ping; Lu, Chun-Hua; Chen, Zhi-Peng; Ge, Feng; Wang, Tong; Wang, Wei; Xiao, Chuan-Le; Yin, Xin-Feng; Liu, Langxia; He, Jian-Xing; He, Qing-Yu	Qing-Yu He	PROTEOMICS	2011	11(3),429-439	3.666
38	Preliminary X-ray crystallographic analysis of the glycosyltransferase from a marine Streptomyces species	Gong, Liping; Xiao, Yi; Liu, Qiang; Li, Sumei; Zhang, Changsheng; Liu, Jinsong	Liu, Jinsong	Acta Crystallographica	2011	67,136-139	0.502

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39	THE EFFECTS OF BACILLI CALMETTE GUERIN-POLYSACCHARIDE NUCLEIC ACID ON NASAL AIRWAY INFLAMMATION AND RESISTANCE IN ALLERGIC RHINITIS MICE	Xi, Y.; Lai, K-F; Han, L-N; Wu, N.; Hong, Y-H; Xie, J-X; Luo, W.; Zhong, N-S	Zhong, N-S	Respirology	2011	16,33-34	2.856
40	ESTABLISHMENT OF REFERENCE VALUES FOR DIFFERENTIAL CELL COUNTS IN NASAL LAVAGE OF HEALTHY YOUNG ADULTS	Xie, Y.; Lai, K.; Xie, J.; Huang, R.; Zhang, Q.; Zhong, N.	Zhong, N	World Allergy Organization Journal	2011	16,37-37	2.856
41	A SAMPLING SURVEY ON RHINITIS AND ITS COMPLICATIONS IN COLLEGE STUDENTS	Xie, Y.; Lai, K.; Xie, J.; Huang, R.; Zhang, Q.; Zhong, N.	Zhong, N	Respirology	2011	16,37-37	2.856
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43	WEIGHT OF CURB-65 FOR COMMUNITY-ACQUIRED PNEUMONIA	Guo, Q.; Li, H-Y; Zhou, Y-P; Li, M.; Chen, X-K; Liu, H.; Peng, H-L; Yu, H-Q; Chen, X.; Liu, N.; Liang, L-H; Zhao, Q-Z; Mei, J.	Mei，J	Respirology	2011	16,50-50	2.856
44	QUADRICEPS DYSFUNCTION RELATES TO MULTIPLE FACTORS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE	Ju, C-R	Ju, C-R	RESPIROLOGY	2011	16,105-105	2.856
45	EFFECTS OF NICOTINE ON STORE- OPERATED CA(2+) INFLUX AND BASAL INTRACELLULAR CA(2+) CONCENTRATION IN RAT DISTAL PULMONARY ARTERIAL SMOOTH MUSCLE CELLS	Chen, Y.; Lu, W.; Ye, Y.; Ou, H.; Zhao, L.; Ran, P.; Zhong, N.; Wang, J.	Wang, J	American Thoracic Society International	2011	16,115-116	2.856

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47	THE EFFECTS OF BACILLI CALMETTE GUERIN (BCG) - POLYSACCHARIDE NUCLEIC ACID (PSN) BY INTRACHACHEAL ADMINSTRATION AT DIFFERENT TIMES ON AIRWAY INFLAMMATION, AIRWAY HYPERRESPONSIVENESS IN ASTHMATIC MOUSE MODEL	Xi, Y.; Lai, K-F; Han, L-N; Jiang, H.; Wu, N.; Hong, Y-H; Zhou, Y-B; Luo, W.; Chen, R-C; Zhong, N-S	Zhou, Y-B	RESPIROLOGY	2011	16,207-208	2.856
48	LEUKOTRIENE D4 BRONCHIAL PROVOCATION TEST IN ASTHMATICS UNDER DIFFERENT CONTROL STATUS	Guan, W.; Gao, Y.; Jiang, C.; An, J.; Yu, X.; Liu, W.; Zheng, J.	Zheng, J	RESPIROLOGY	2011	16,210-210	2.856
49	Copper-Catalyzed Intramolecular Dehydrogenative Aminooxygenation: Direct Access to Formyl-Substituted Aromatic N-Heterocycles	Wang, Honggen; Wang, Yong; Liang, Dongdong; Liu, Lanying; Zhang, Jiancun; Zhu, Qiang	Zhang, JC	ChemInform	2011	50(25),5677-5680	12.111
50	Discovery of highly potent agents against influenza A virus	Zhao, Xin; Li, Chufang; Zeng, Shaogao; Hu, Wenhui	Hu, Wenhui	European Journal of Medicinal Chemistry	2011	46(1),52-57	3.982
51	Phosphoproteome profile of human lung cancer cell line A549	Yu, Guangchuang; Xiao, Chuan-Le; Lu, Chun-Hua; Jia, Hai-Tao; Ge, Feng; Wang, Wei; Yin, Xing-Feng; Jia, Hong-Ling; He, Jian-Xing; He, Qing-Yu	He, JX	Molecular Biosystems	2011	7(2),472-479	2.807

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53	miR-106a-mediated Malignant Transformation of Cells Induced by Anti-benzo[a]pyrene-trans-7,8-diol-9,10-epoxide	Jiang, Yiguo; Wu, Yan; Greenlee, Anne R.; Wu, Jianjun; Han, Zhiyuan; Li, Xun; Zhao, Yao	Jiang, YG	Toxicological Sciences An Official Journal of the Society of Toxicology	2011	119(1),50-+	4.307
54	Comparative immunogenicity of recombinant adenovirus-vectored vaccines expressing different forms of hemagglutinin (HA) proteins from the H5 serotype of influenza A viruses in mice	Hu, Xiangjing; Meng, Weixu; Dong, Zhenyuan; Pan, Weiqi; Sun, Caijun; Chen, Ling	Chen, L	Virus Research	2011	155(1),156-162	2.611
55	COMPARISON OF LEUKOTRIENE D4 AND METHACHOLINE BRONCHIAL PROVOCATION TEST FOR SCREENING ASTHMATICS: AN EFFECTIVENESS AND SAFETY ANALYSIS	Guan, W.; Gao, Y.; Jiang, C.; An, J.; Yu, X.; Liu, W.; Zheng, J.	Zheng, J	RESPIROLOGY	2011	16,210-210	2.856
56	ACUTE HYPOXIA INCREASES INTRACELLULAR CA(2+) CONCENTRATION VIA ENHANCEMENT OF STORE-OPERATED CA(2+) ENTRY IN RAT DISTAL PULMONARY VENOUS SMOOTH	Peng, G.; Ran, P.	Ran, P	RESPIROLOGY	2011	16,304-304	2.856

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57	THE MECHANISM OF ANTI-PULMONARY FIBROSIS OF HUMAN INDUCED HUMAN CD4(+)CD25(+)FOXP3(+) CELLS	Wang, J.; Xu, J.	Xu, J	RESPIROLOGY	2011	16,322-322	2.856
58	CONSTRUCTION OF INDUCIBLE SHRNA EXPRESSION VECTORS FOR HMGB1 AND APPLICATION IN MICE MODEL OF PULMONARY FIBROSIS	Wang, Y.; Zhou, B.; Luo, Y.; Xu, J.	Zhou, B	RESPIROLOGY	2011	16,322-322	2.856
59	COMPARISON OF MOUSE PULMONARY FIBROSIS MODELS INDUCED BY BLEOMYCIN WITH DIFFERENT STRAIN OF MICE	Tan, Y.; Liu, R.; Chen, G.; Li, H.; Zhao, J.	Zhao, J	RESPIROLOGY	2011	16,324-324	2.856
60	The Polymorphism and Haplotypes of PIN1 Gene are Associated with the Risk of Lung Cancer in Southern and Eastern Chinese Populations	Lu, Jiachun; Yang, Lei; Zhao, Hongjun; Liu, Bin; Li, Yinyan; Wu, Hongxia; Li, Qingchu; Zeng, Bohang; Wang, Yunnan; Ji, Weidong; Zhou, Yifeng	Lu, JC	HUMAN MUTATION	2011	32(11),1299-1308	4.937
61	5-Ethynyl-2'-deoxyuridine as a molecular probe of cell proliferation for high-content siRNA screening assay by "click" chemistry	Chen MiaoJuan; Qu DeZhong; Chi WeiLin; Wang Wei; Ren XiaoShuai; Cong ShuJie; Liang PeiZhou; Feng ShiPeng; Zhang BiLiang	Zhang, B L	Science China-Chemistry	2011	54(11),1702-1710	1.652
62	Nobiletin, a Polymethoxylated Flavonoid from Citrus, Shows Anti-Angiogenic Activity in a Zebrafish In Vivo Model and HUVEC In Vitro Model	Lam, Kai Heng; Alex, Deepa; Lam, In Kei; Tsui, Stephen Kwok Wing; Yang, Zi Feng; Lee, Simon Ming Yuen	Lee, S M Y	JOURNAL OF CELLULAR BIOCHEMISTRY	2011	112(11),3313-3321	3.155

63	Heat Shock Protein-60 Expression was Significantly Correlated With the Prognosis of Lung Adenocarcinoma	Xu, Xin; Wang, Wei; Shao, Wenlong; Yin, Weiqiang; Chen, Hanzhang; Qiu, Yuan; Mo, Mingcong; Zhao, Jin; Deng, Qiuhua; He, Jianxing	He, J X	JOURNAL OF SURGICAL ONCOLOGY	2011	104(6),598-603	2.888
64	RNAi targeting of hTERT gene expression induces apoptosis and inhibits the proliferation of lung cancer cells	Ge, Linhu; Shao, Wenlong; Zhang, Yangde; Qiu, Yuan; Cui, Dong; Huang, Donghai; Deng, Zhansheng	Deng, Z S	Oncology Letters	2011	2(6),1121-1129	1.448
65	Expression, purification, and refolding of active human and mouse secreted group IIE phospholipase A(2)	Zhang, Yujie; Xu, Tingting; Chen, Qiujia; Wang, Bing; Liu, Jinsong	Liu, J S	PROTEIN EXPRESSION AND PURIFICATION	2011	80(1),68-73	1.401
66	A Common Genetic Variant (97906C > A) of DAB2IP/AIP1 Is Associated with an Increased Risk and Early Onset of Lung Cancer in Chinese Males	Yang, Lei; Li, Yinyan; Ling, Xiaoxuan; Liu, Lin; Liu, Bin; Xu, Kevin; Bin, Xiaonong; Ji, Weidong; Lu, Jiachun	Yang, L	PLoS One	2011	6(10)	3.535
67	Towards improving the diagnosis and management of chronic cough in China	Zeng Guang-qiao; Sun Bao-qing; Zhong Nan-shan	Zhong, N S	CHINESE MEDICAL JOURNAL	2011	124(20),3205-3206	1.017
68	The Chinese national guidelines on diagnosis and management of cough (December 2010)	Lai Ke-fang	Lai, K F	CHINESE MEDICAL JOURNAL	2011	124(20),3207-3219	1.017
69	Inhibition of enterovirus 71 replication by chrysosplenetin and penduletin	Zhu, Qin-Chang; Wang, Yi; Liu, Ya-Ping; Zhang, Rui-Qi; Li, Xiang;	Peng, T	EUROPEAN	2011	44(3),392-398	3.752

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70	Organocatalytic Asymmetric Domino Aza-Michael-Mannich Reaction: Synthesis of Tetrahydroimidazopyrimidine Derivatives	Li, Hong; Zhao, Junling; Zeng, Lili; Hu, Wenhui	Zhao, J L	JOURNAL OF ORGANIC CHEMISTRY	2011	76(19),8064-8069	4.538
71	Bronsted Acid-Promoted Sequential Hydroarylation-Hydroamidation of Arene-Tethered 1-(2-Alkynylphenyl)ureas: Direct Access to 4,4-Spiro-3,4-dihydro-2-(1H)-quinazolinones	Wang, Honggen; Zhao, Jiaji; Zhang, Jiancun; Zhu, Qiang	Zhang, J C	ADVANCED SYNTHESIS & CATALYSIS	2011	353(14-15),2653-2658	5.852
72	Antimalarial beta-Carboline and Indolactam Alkaloids from Marinactinospora thermotolerans, a Deep Sea Isolate	Huang, Hongbo; Yao, Yueliang; He, Zhengxiang; Yang, Tingting; Ma, Junying; Tian, Xinpeng; Li, Yayong; Huang, Caiguo; Chen, Xiaoping; Li, Wenjun; Zhang, Si; Zhang, Changsheng; Ju, Jianhua	Ju, J H	JOURNAL OF NATURAL PRODUCTS	2011	74(10),2122-2127	3.644
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74	Identification and characterization of acyclovir-resistant clinical HSV-1 isolates from children	Wang, Yi; Wang, Qi; Zhu, Qinchang; Zhou, Rong; Liu, Jinsong; Peng, Tao	Liu, J S	JOURNAL OF CLINICAL VIROLOGY	2011	52(2),107-112	2.882
75	Outdoor air pollution and respiratory health in Asia	Chung, Kian Fan; Zhang, Junfeng; Zhong, Nanshan	Chung, K A F	RESPIROLOGY	2011	16(7),1023-1026	2.856
76	LAMP-based method for a rapid identification of Legionella spp. and Legionella pneumophila	Lu, Xi; Mo, Zi-Yao; Zhao, Hong-Bo; Yan, He; Shi, Lei	Mo, Z Y	APPLIED MICROBIOLOGY AND BIOTECHNOLOGY	2011	92(1),179-187	3.882
77	Weight of the IDSA/ATS minor criteria for severe community-acquired pneumonia	Guo, Qi; Li, Hai-yan; Zhou, Yi-ping; Li, Ming; Chen, Xiao-ke; Liu, Hui; Peng, Hong-lin; Yu, Hai-qiong; Chen, Xia; Liu, Nian; Liang, Li-Hua; Zhao, Qing-zhou; Jiang, Mei	Guo, Q	RESPIRATORY MEDICINE	2011	105(10),1543-1549	3.213
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81	The role of miR-506 in transformed 16HBE cells induced by anti-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide	Zhao, Yao; Liu, Huanying; Li, Yuanqi; Wu, Jianjun; Greenlee, Anne R.; Yang, Chengfeng; Jiang, Yiguo	Jiang, Y G	TOXICOLOGY LETTERS	2011	205(3),320-326	3.571
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83	Palladium-Catalyzed Intramolecular C(sp(2))-H Amidination by Isonitrile Insertion Provides Direct Access to 4-Aminoquinazolines from N-Arylamidines	Wang, Yong; Wang, Honggen; Peng, Jiangling; Zhu, Qiang	Zhu, Q	ORGANIC LETTERS	2011	13(17),4604-4607	6.033

84	Construction and characterization of human adenovirus serotype 3 packaged by serotype 7 hexon	Tian, Xingui; Su, Xiaobo; Li, Haitao; Li, Xiao; Zhou, Zhichao; Liu, Wenkuan; Zhou, Rong	Zhou, R	VIRUS RESEARCH	2011	160(42371),214-220	2.611
85	The phenotype and function of naturally existing regulatory dendritic cells in nematode-infected mice	Li, Zhaotao; Liu, Guiyun; Chen, Yue; Liu, Yunfeng; Liu, Boyu; Su, Zhong	Su, Z	INTERNATIONAL JOURNAL FOR PARASITOLOGY	2011	41(11),1129-1137	3.665
86	Quadriceps strength assessed by magnetic stimulation of femoral nerve in patients with chronic obstructive pulmonary disease	Ju Chun-rong; Chen Rong-chang	Chen, R C	CHINESE MEDICAL JOURNAL	2011	124(15),2309-2315	1.017
87	Synthesis of Quinazolin-4(3H)-ones via Pd(II)-Catalyzed Intramolecular C(sp(2))-H Carboxamidation of N-arylamidines	Ma, Bin; Wang, Yong; Peng, Jiangling; Zhu, Qiang	Zhu, Q	JOURNAL OF ORGANIC CHEMISTRY	2011	76(15),6362-6366	4.538
88	Transition of tumor-associated macrophages from MHC class IIhi to MHC class IIlow mediates tumor progression in mice	Wang, Benfan; Li, Qinyan; Qin, Li; Zhao, Siting; Wang, Jinyan; Chen, Xiaoping	Wang, J Y	BMC IMMUNOLOGY	2011	12	2.594
89	MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells	Feng, Shipeng; Cong, Shujie; Zhang, Xin; Bao, Xichen; Wang, Wei; Li, Huiping; Wang, Zhe; Wang, Guoxin; Xu, Jianzhen; Du, Bowen; Qu, Dezhong; Xiong, Wei; Yin, Menghui; Ren, Xiaoshuai; Wang, Feifei;etc	Zhang, B L	NUCLEIC ACIDS RESEARCH	2011	39(15),6669-6678	8.647

90	Long-term outcome of hybrid surgical approach of video-assisted minithoracotomy sleeve lobectomy for non-small-cell lung cancer	He, Jianxing; Shao, Wenlong; Cao, Christopher; Yan, Tristan D.; Wang, Daoyuan; Xiong, Xinguo; Yin, Weiqiang; Xu, Xin; Huang, Jun	He, J X	SURGICAL ENDOSCOPY AND OTHER INTERVENTIONAL TECHNIQUES	2011	25(8),2509-2515	3.499
91	Long-Term Outcome and Cost-Effectiveness of Complete Versus Assisted Video-Assisted Thoracic Surgery for Non-Small Cell Lung Cancer	He, Jianxing; Shao, Wenlong; Cao, Christopher; Yan, Tristan; Wang, Daoyuan; Xiong, Xin-Guo; Yin, Weiqiang; Xu, Xin; Chen, Hanzhang; Qiu, Yuan; Zhong, Baoliang	He, J X	JOURNAL OF SURGICAL ONCOLOGY	2011	104(2),162-168	2.888
92	Detection of CD4(+)CD25(+)FOXP3(+) regulatory T cells in peripheral blood of patients with chronic autoimmune urticaria	Sun, Ren-Shan; Sui, Jian-Feng; Chen, Xiao-Hong; Ran, Xin-Ze; Yang, Zi-Feng; Guan, Wen-Da; Yang, Tao	Sun, R S	AUSTRALASIAN JOURNAL OF DERMATOLOGY	2011	52(3),E15-E18	1.263
93	A genome-wide association study identifies two new lung cancer susceptibility loci at 13q12.12 and 22q12.2 in Han Chinese	Hu, Zhibin; Wu, Chen; Shi, Yongyong; Guo, Huan; Zhao, Xueying; Yin, Zhihua; Yang, Lei; Dai, Juncheng; Hu, Lingmin; Tan, Wen; Li, Zhiqiang; Deng, Qifei;	Shen, H B	NATURE GENETICS	2011	43(8),792-U103	32.197

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95	NF kappa B1 and NF kappa BIA Polymorphisms Are Associated with Increased Risk for Sporadic Colorectal Cancer in a Southern Chinese Population	Song, Shunxin; Chen, Dianke; Lu, Jiachun; Liao, Jiawei; Luo, Yanxin; Yang, Zuli; Fu, Xinhui; Fan, Xinjuan; Wei, Yisheng; Yang, Lei; Wang, Lei; Wang, Jianping	Song, S X	PLoS One	2011	6(6)	3.535
96	Mechanistic insights into the roles of three linked single-stranded template binding residues of MMLV reverse transcriptase in misincorporation and mispair extension fidelity of DNA synthesis	Xie, Jian; Zhang, Pengwei; Li, Chuanjiang; Huang, Qianhua; Zhou, Rong; Peng, Tao	Zhou, R	GENE	2011	479(42371),47-56	2.258
97	Antimalarial Effects of Human Immunodeficiency Virus Protease Inhibitors in Rhesus Macaques	Li, Youjia; Qin, Li; Peng, Nanzheng; Liu, Guangjie; Zhao, Siting; He, Zhengxiang; Chen, Xiaoping	Chen, X P	ANTIMICROBIAL AGENTS AND CHEMOTHERAPY	2011	55(6),3039-3042	4.547
98	Sequential Hydration-Condensation-Double Cyclization of Pyridine-Substituted 2-Alkynylanilines: An Efficient Approach to Quinoline-Based Heterocycles	Peng, Lijie; Wang, Honggen; Peng, Changlan; Ding, Ke; Zhu, Qiang	Ding, K,Zhu, Q	SYNTHESIS-STUTTGART	2011	(11),1723-1732	2.348
99	Indoor allergen levels in Guangzhou city, southern	Lai, X.; Zhang, C.; Gjesing, B.; Li, J.;	Lai, X	ALLERGY	2011	66,127-	5.746

	China	Zhong, N.; Spangfort, M.				127	
100	Specific IgE prevalence and cross-reactivity of house dust mites and storage mites in asthmatic children from Haikou, China	Zheng, Y.; Chen, S.; Lai, X.; Gjesing, B.; Zhong, N.; Spangfort, M.	Spangfort, M	ALLERGY	2011	66,330-330	5.746
101	Epidemiology of adenovirus type 5 neutralizing antibodies in healthy people and AIDS patients in Guangzhou, southern China	Sun, Caijun; Zhang, Yinfeng; Feng, Liqiang; Pan, Weiqi; Zhang, Maochao; Hong, Zheyu; Ma, Xin; Chen, Xiaoping; Chen, Ling	Sun, C J	VACCINE	2011	29(22),3837-3841	3.338
102	Immunogenicity and Safety of a China-Made Monovalent Pandemic (H1N1) 2009 Influenza A Vaccine in Healthcare Workers in Guangzhou, China	Zhan, Yangqing; Yang, Zifeng; Li, Lianna; Ye, Dan; Wu, Huiyan; Fu, Renzhen; Zhao, Suishan; Wang, Yutao; Zhou, Rong; Chen, Rongchang	Chen, R C	JAPANESE JOURNAL OF INFECTIOUS DISEASES	2011	64(3),190-194	1.249
103	Inactivation of Rac1 reduces Trastuzumab resistance in PTEN deficient and insulin-like growth factor I receptor overexpressing human breast cancer SKBR3 cells	Zhao, Yong; Wang, Zhishan; Jiang, Yiguo; Yang, Chengfeng	Yang, C F	CANCER LETTERS	2011	313(1),54-63	5.174
104	Allergen micro-array detection of specific IgE-reactivity in Chinese allergy patients	Zheng Yi-wu; Li Jing; Lai Xu-xin; Zhao De-yu; Liu Xiao-fan; Lin Xiao-ping; Gjesing, Birgitte; Palazzo, Paola; Mari, Adriano; Zhong Nan-shan; Spangfort, Michael D.	Zheng, Y W	CHINESE MEDICAL JOURNAL	2011	124(24),4350-4354	1.017
105	Effects of dead space loading on neuro-muscular and neuro-ventilatory coupling of the respiratory system during exercise in healthy adults: Implications for	Jensen, Dennis; O'Donnell, Denis E.; Li, Ruifa; Luo, Yuan-Ming	Luo, Y M	RESPIRATORY PHYSIOL	2011	179(42403),219-226	1.916

	dyspnea and exercise tolerance			OGY & NEUROBIOLOGY			
106	Low-level expression of let-7a in gastric cancer and its involvement in tumorigenesis by targeting RAB40C	Yang, Qiaoyuan; Jie, Zhigang; Cao, Hong; Greenlee, Anne R.; Yang, Chengfeng; Zou, Fei; Jiang, Yiguo	Jiang, Y G	CARCINOGENESIS	2011	32(5),713-722	5.368
107	The Role of NF-E2-Related Factor 2 in Predicting Chemoresistance and Prognosis in Advanced Non-Small-Cell Lung Cancer	Yang, Haihong; Wang, Wei; Zhang, Yalei; Zhao, Jin; Lin, Enyun; Gao, Jing; He, Jianxing	He, J X	Clinical Lung Cancer	2011	12(3),166-171	2.866
108	The specific IgE reactivity pattern of weed pollen-induced allergic rhinitis patients	Han, Demin; Lai, Xuxin; Gjesing, Birgitte; Zhong, Nanshan; Zhang, Luo; Spangfort, Michael D.	Zhang, L	ACTA OTO-LARYNGOLOGICA	2011	131(5),533-538	1.218
109	Crystal structure of E339K mutated human glucokinase reveals changes in the ATP binding site	Liu, Qiang; Shen, Yunfeng; Liu, Sanling; Weng, Jianping; Liu, Jinsong	Liu, J S	FEBS LETTERS	2011	585(8),1175-1179	3.478
110	Practical one-pot protocol for the syntheses of 2-chloro-pyrrolo[3,2-d]pyrimidines	Zhao, Liwen; Tao, Kemei; Li, Haitian; Zhang, Jiancun	Zhang, Jiancun	《ChemInform》	2011	67(15),2803-2806	2.621
111	Structure Identification of Euphorbia Factor L3 and Its Induction of Apoptosis through the Mitochondrial Pathway	Zhang, Jian-Ye; Liang, Yong-Ju; Chen, Hu-Biao; Zheng, Li-Sheng; Mi, Yan-Jun; Wang, Fang; Zhao, Xiao-Qin; Wang, Xiao-Kun; Zhang, Hui; Fu, Li-Wu	Zhang, J Y	MOLECULES	2011	16(4),3222-3231	2.749
112	Cough reflex sensitivity is increased in guinea pigs with parainfluenza virus infection	Ye, X. M.; Zhong, N. S.; Liu, C. L.; Chen, R. C.	Zhong, N S	EXPERIMENTAL	2011	37(3),186-194	1.419

				LUNG RESEARCH			
113	Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern China	Liu, Wen-Kuan; Chen, De-Hui; Liu, Qian; Liang, Huan-Xi; Yang, Zi-Feng; Qin, Sheng; Zhou, Rong	Zhou, R	BMC INFECTIOUS DISEASES	2011	11	2.864
114	Potent Neutralization of Influenza A Virus by a Single-Domain Antibody Blocking M2 Ion Channel Protein	Wei, Guowei; Meng, Weixu; Guo, Haijiang; Pan, Weiqi; Liu, Jinsong; Peng, Tao; Chen, Ling; Chen, Chang-You	Wei, G W	PLoS One	2011	6(12)	3.535

第二部分：实验室人才队伍建设

1、 陶爱林教授
2、 谭守勇教授
3、 张清玲博士
4、 张海波教授
5、 丁克博士
6、 赵金存博士

陶爱林，男，教授。现任广州市过敏反应与临床免疫重点实验室主任、教授、硕导，国家转基因安全评价委员会委员，广东省医学会变态反应学分会委员。围绕低过敏原性过敏原改良与鉴定、食品药品的过敏原性评价与改良开展了一些列研究，提出了“代表性过敏原”及“光谱性免疫调节剂”等学术新概念和“抗原平衡刺激”的假设；专注于过敏疾病诊治、过敏原及食品药品过敏原性的医学评价与改良，创建了抗原性（过敏原性）评价与改良的技术体系，一例低过敏原性靶向性免疫毒素已经申请国际发明专利，多例业经低过敏原性改造的过敏原已经申请国家发明专利；在创建新算法的基础上，开发了过敏原生物信息学判别软件 SORTALLER，该软件不仅运行速度快，而且能同时进行多序列判别，更重要的是其准确性国际领先，已经挂网运行（http://sortaller.gzhmc.edu.cn）。组织 30 多篇论文在 SCI 及国内核心期刊发表，国际国内会议论文 20 多篇（其中 2 篇被 ISTP 收录），大会报告多次。获得国家发明专利 2 项。

谭守勇，男，主任医师，中山大学临床兼职教授，院长，中山大学医学硕士，从事结核病科及呼吸内科临床、科研、教学工作 20 多年，擅长于结核病的诊断与鉴别诊断及难治肺结核的治疗工作。发表专业论文 30 余篇。任中华医学会结核病学分科学会委员；中国防痨协会理事、临床专业组委员；广东省医学会结核病分科学会主任委员；广东省防痨协会副会长；广东省医学会呼吸病学分会常务委员；广东省医师协会呼吸专业组常务委员；广州市医学会结核病分科学会主任委员；广东省医师协会人文医学工作委员会副主任委员；广州市医师协会副会长。广州医学院硕士研究生导师；中山大学硕士研究生导师。研究方向有肺结核继发肺真菌感染的诊治进展，结核病及呼吸系统疾病等。

张清玲，女，博士，从事呼吸内科专业。原为深圳市第二人民医院呼吸内科副主任医师，后赴英国帝国理工大学医学院心肺疾病研究所（National Heart & Lung institute, Imperial College），曾获国家自然基金、广东省自然科学基金、广东省科技攻关项目等。

张海波，男，加拿大多伦多大学教授，主要从事呼吸内科、免疫学、生理学及危重病学。急性肺损伤及重症感染这一领域的基础研究一直是国际难题，也是呼吸疾病国家重点实验室大力支持的研究方向之一。张海波教授是国际著名免疫学、生理学及危重病学专家，在感染,免疫和急性肺损伤研究方面贡献突出，国际公认。目前在国际学术期刊上发表 SCI 论文 160 余篇，影响因子累计达 700 余分。

作为广州医学院的客座教授，他于 2011 年开始与所内多位专家合作，创建了转化医学实验室。旨在借助多伦多大学在危重医学及呼吸系统疾病研究领域的世界领先地位，将国际前沿研究方向和先进的技术方法引进并融入到转化实验室，运用临床资源研究急性肺损伤及重症感染机理，强化基础研究，推动转化医学，促进呼吸疾病的基础与临床有机结合，加强该领域的发病机制与防治研究。

在过去一年，张海波教授带领各课题组成员取得了初步成绩：首先，积极组织 2012 年度国家自然科学基金科研项目的申报工作，对申报项目的人员进行一对一指导；其次，加强转化医学科研平台建设，借鉴多伦多大学的经验逐步开展危重医学临床资源库的建设；再次，联合培养优秀青年人才，通过选派科研人员赴加拿大多伦多大学学习，培养具有国际先进水平的呼吸系统疾病转化医学研究领域的优秀青年学者；最后，定期召开课题组工作会议，对实验结果进行讨论、分析、指导，并积极开展国际学术交流活动。

丁克，中科院广州生物医药与健康研究院研究员，博士生导师。2005 年 3 月任美国密西根大学医学院 Research Inivestigator。2006 年 3 月回国加入中科院广州生物医药与健康研究院， 2007 年入选“中国科学院百人计划”。2008 年入选广州市优秀专家。2009 年获“广东省丁颖科技奖”。现任中科院广州生物医药与健康研究院研究员，中科院广州生物医药与健康研究院化学生物所副所长。

科技部公布了 2014 年创新人才推进计划入选名单，丁克入选中青年科技创新领军人才计划。

丁克的研究工作主要集中在新型抗肿瘤药物、治疗代谢性疾病药物等具有重要生物活性的小分子调节剂的设计和合成，为新药开发提供先导化合物。成功设计合成了：1）Spirooxindole 类 MDM2 抑制剂（以 3.98 亿美元转让 Sanifi-Aventis)；2）异黄酮类 Bcl-2 抑制剂(转让美国 Ascenta，其中 AT-101 已进入 II 期临床)；3）世界首个选择性 ERRa 激动剂；4）可有效克服格列卫临床耐受的全新蛋白激酶抑制剂等；5）首次发现了 niclosamide 为全新的 STAT3 抑制剂；6）开发了 quinazolinone，取代吲哚，取代 indazole 等的高效合成方法。

丁克在国际期刊如“J. Am. Chem. Soc.”、“J. Med. Chem.”、“Chem. Commun”、“J. Org. Chem.”、“Org. Letts”等发表论文 60 多篇，并申请国际、国家专利近 30 余项。2010 年任 ACS Med. Chem. Lett.顾问编委。

赵金存，1978 年 8 月出生于天津。2002 年本科毕业于北京大学医学部基础医学系，2007 年获北京大学医学部免疫学系免疫学博士；2007 年 8 月至 2012 年 8 月于美国爱荷华大学微生物系开展博士后研究；2012 年 9 月任美国爱荷华大学微生物系助理研究科学家（Assistant Research Scientist）。赵金存主要关注于呼吸系统疾病及其致病病毒致病的免疫学机制研究，近年来在 J Clin Invest、J Exp Med、Proc Natl Acad Sci U S A、PLoS Pathog、J Immunol、J Virol 等杂志发表论文近 30 篇。赵金存主要研究领域为呼吸道冠状病毒等，以第一作者在 Journal of Clinical Investigation, PNAS, PloS Pathogens, Journal of Virology 等著名杂志上发表多篇论文。

赵金存于 11 年前在北京参与了 SARS 冠状病毒的研究，2007 赴美国继续研究冠状病毒，近年投入了中东新发冠状病毒的研究，并将于 2015 年年底将全职加入广州医科大学/广州呼吸疾病研究所/呼吸疾病国家重点实验室工作。

第三部分：实验室对外交流

本年度国内外学者来访一览表：

学者姓名	讲学/研究题目	学者类别	单位
Larissa Shimoda	肺动脉高压发病机制的研究	讲学	约翰·霍普金斯大学
Dr. Xinhua Ji	Structure and Functional Cycle of Essential GTPase Era:Implications for Ribosome Biogenesis and Antibiotic Discovery	讲学	Macromolecular Crystallography Laboratory,Frederick, MD, USA
Fan Shenying	GWAS 的统计学方法	讲学	美国德克萨斯大学
周翊峰	科研合作	讲学	苏州大学
张正东	国家基金申报技巧	讲学	南京医科大学
陈哲声	肿瘤耐药机制	讲学	美国圣约翰大学
杨静	磷酸化信号基因与白血病治疗	讲学	美国德克萨斯大学
陈敬贤	病毒学技术	讲学	--
Peter Krell	腺病毒、流感病毒分子生物学及其疫苗的研究进展	讲学	--
Donald Seto	Confluence of genomics/bioinformatics and information technology: Applications to adenoviruses (and Paradigm Shifts)	讲学	美国乔治梅森大学系统生物学院生物信息学和计算生物学系
王敏秀	流感病毒的入侵及治疗	讲学	--
韦妙宜	腺病毒与糖尿病和肥胖病的关系	讲学	--
高为	基因组学在急性肺损伤和肺动脉高压中的应用	讲学	约翰霍普金丝大学哮喘与变态反应学中心
孙兴国	美国胸科医师协会肺功能临床解读	讲学	美国加州大学洛杉矶分校
Surinder S Birring	慢性咳嗽研究现状	讲学	--
李铭源	如何应用现代分子生物学技术研究中药	讲学	澳门大学中华医药研究所

学者姓名	讲学/研究题目	学者类别	单位
Katsuhiro Konno	日本中药(汉方)研究现状	讲学	日本富山大学
Peter Hylands	Recent studies on TCM	讲学	英国 King's College

本年度实验室人员主要参会交流一览表：

会议名称	地点	时间	报告人	类型	题目
GSK 国际呼吸会议	广州	2011 年 3 月 1 日	郑劲平	大会发言	COPD 的抗炎治疗进展
国际 COPD 呼吸会议(广州)	广州	2011 年 3 月 29 日	郑劲平	大会发言	Difference of airway reversibility assessment between FEV1 change as percent predicted and change from baseline in COPD
第三届中国小核酸技术与应用学术会议	昆山	2011 年 4 月 26 日	张必良	大会发言	miRNA 可用于肺癌治疗吗
香港医师论坛	香港	2011 年 5 月 8 日	郑劲平	大会发言	Impact of Smoking Cessation on Respiratory Health
中国毒理学会第三届中青年学者科技论坛	上海	2011 年 5 月 17 日	蒋义国	大会发言	化学物诱导细胞癌变中 microRNA 致癌和抗癌机制
中日英咳嗽专家论坛	北京	2011 年 5 月 20 日	赖克方	大会发言	慢性咳嗽治疗进展
第 11 届中国科技期刊青年编辑学术研讨会	广州	2011 年 5 月 23 日	曾广翘	大会发言	关于 SCI 论文投稿的演讲
上海市胸科医院交流	上海	2011 年 6 月 1 日	郑劲平	大会发言	肺功能检查—昨天今天和明天
广东省变态反应疾病学会年会	中山	2011 年 6 月 12 日	郑劲平	大会发言	支气管激发试验的研究进展
第七届 SINO-US 华人化学教授会议	贵州	2011 年 6 月 27 日	朱强	大会发言	Novel Accesses to Several N-Heterocycles
第八届中青年呼吸医师论坛	银川	2011 年 7 月 8 日	赖克方	大会发言	嗜酸粒细胞性支气管炎及其与哮喘的关系
The 6th Korea-China Symposium on Organic Chemistry	韩国	2011 年 7 月 18 日	朱强	大会发言	Transition-metal-catalyzed C?H Functionalization in the Synthesis of N-Heterocycles
第五届中国咳嗽论坛	北京	2011 年 7 月 26 日	赖克方	大会发言	2011 年咳嗽研究年度进展
中国呼吸医师论坛	洛阳	2011 年 8 月 4 日	陈荣昌	大会发言	如何更好地开展无创通气
肺发育、损伤修复和再生”国际研讨会	广州	2011 年 8 月 7 日	王健	大会发言	Role of BMP4 in hypoxic pulmonary arterial hypertension
肺发育、损伤修复和再生”国际研讨会	广州	2011 年 8 月 7 日	卢文菊	大会发言	Bone Morphogenetic Protein 4 Enhanced Resting [Ca2+]i,

					and Canonical Transient Receptor Potential Expression in Pulmonary Arterial Smooth Muscle Cells
Grover Conference	美国	2011 年 9 月 9 日	王健	大会发言	Sildenafil-cGMP-PKG-PPARgamma Signaling Pathway Inhibits Angiotensin-II Induced Transient Receptor Potential Canonical (TRPC) 6 Expression In Pulmonary Arterial Smooth Muscles Cells
Grover Conference	美国	2011 年 9 月 9 日	卢文菊	大会发言	Sildenafil-cGMP-PKG-PPARgamma Signaling Pathway Inhibits Angiotensin-II Induced Transient Receptor Potential Canonical (TRPC) 6 Expression In Pulmonary Arterial Smooth Muscles Cells.
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	冉丕鑫	大会发言	适宜社区和基层应用的 COPD 初步筛选技术研究
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	赖克方	大会发言	慢性咳嗽的诊治新进展
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	陈荣昌	大会发言	社区获得性呼吸道感染规范化治疗策略
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	卢文菊	大会发言	丹参酮ⅡA 磺酸钠抑制低氧性肺动脉高压大鼠模型 IL-6 的表达
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	郑劲平	大会发言	我国成人肺功能正常值及预计方程式建立
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	罗远明	大会发言	COPD 患者的睡眠异常
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	黎毅敏	大会发言	体外膜肺氧合临床应用操作与体会
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	卓超	大会发言	金黄色葡萄球菌万古霉素 MIC 漂移研究
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	高怡	大会发言	用力肺功能检查的不良反应分析
中华医学会第十二次全国呼吸病学术会议	广州	2011 年 9 月 15 日	陈愉	大会发言	局部注射曲安奈德结核常规介入方法治疗难治性良性中央气道狭窄的疗效和安全性研究
2011 年全国医院情报图书管理学术会议	广州	2011 年 9 月 16 日	曾广翘	大会发言	成功发表 SCI 论文的战略与战术

Asian3 2011 on Nucleic Acid (中日韩三国核酸会议）	武汉	2011 年 10 月 14 日	张必良	大会发言	Using Click Chemistry to Label Cellular DNA and RNA
第九届江淮有机化学论坛	常州(常州大学)	2011 年 10 月 14 日	朱强	大会发言	Transition-metal-catalyzed C?H Functionalization in the Synthesis of Heterocycles
2011 年美国胸科医师年会	美国夏威夷	2011 年 10 月 21 日	孙宝清	大会发言	The Prevalence of Sensitivity to Cockroach Allergens and IgE Cross-Reactivity Between Cockroach and House Dust Mite Allergens in Chinese Patients With Allergic Rhinitis and Asthma.
2011 年美国胸科医师年会	美国夏威夷	2011 年 10 月 21 日	郑劲平	大会发言	COPD in developing Countries
SINO-FRENCH BILATERAL GREEN CHEMISTRY CONFERENCE	广州(华南理工大学)	2011 年 10 月 31 日	朱强	大会发言	Transition-metal-catalyzed C?H Functionalization in the Synthesis of Heterocycles
2011 年亚太呼吸年会	上海	2011 年 11 月 3 日	罗远明	大会发言	neural drive of Diaphragm and upper airway muscles in sleep apnoea
2011 年亚太呼吸年会	上海	2011 年 11 月 3 日	赖克方	大会发言	Lower airway inflammation and hyperresponsiveness in allergic and non-allergic rhinitis …
2011 年亚太呼吸年会	上海	2011 年 11 月 3 日	陈荣昌、巨春蓉	大会发言	Serum myostatin levels and nutritional status in patients with chronic obstructive pulmonary disease.
2011 年亚太呼吸年会	上海	2011 年 11 月 3 日	陈荣昌、顾为丽	大会发言	Clinical outcomes of a novel breathing training manoeuvre in patients with COPD.
2011 年亚太呼吸年会	上海	2011 年 11 月 3 日	卢文菊	大会发言	Effects of nicotine on store-operated Ca2+ influx and basal intracellular Ca2+ concentration in rat distal pulmonary arterial smooth muscle cells
2011 年亚太呼吸年会	上海	2011 年 11 月 3 日	张清玲	大会发言	Disturb Microbiome in the airways of Severe
2011 年亚太呼吸年会	上海	2011 年 11 月 6 日	郑劲平	大会发言	importance of reference values in lung function tests

中国化学会第七届全国有机化学学术会议	南京	2011 年 11 月 12 日	朱强	大会发言	Transition-metal-catalyzed C?H Functionalization in the Synthesis of Heterocycles
第二届岭南有机化学论坛,	广州(中山大学)	2011 年 11 月 27 日	朱强	大会发言	Transition-metal-catalyzed C?H Functionalization in the Synthesis of Heterocycles
全球华人临床微生物及感染症学术论坛	台北	2011-10-29 至 2011-10-31	卓超	大会发言	临床重症感染的治疗问题
转基因作物和新蛋白安全评价” 研讨会	北京	2011-11-07 至 2011-11-08	陶爱林	大会发言	题目不详
Experiment Biology	华盛顿	2011-4-12 至 13 日	王健	大会发言	Knockdown of canonical transient receptor potential proteins 1, 4, and 6 attenuates store-operated calcium entry and calcium responses to acute hypoxia in pulmonary arterial smooth muscle cell
Experiment Biology	华盛顿	2011-4-12 至 13 日	卢文菊	大会发言	Chronic Hypoxia increases Orai1 and Orai2 Expression in Pulmonary Arterial Smooth Muscle
Lancet Oncology			何建行	大会发言	Video-Assisted Thoracic Surgery Lobectomy for Non-Smal Cell lung Cancer.

第四部分：科研团队工作进展

一、 哮喘与咳嗽团队

团队今年新获科研项目 9 项。其中国家重大科技专项 1 项，国自然面上项目 2 项，国自然青年基金 2 项，国自然国际合作交流项目 1 项，省级项目 2 项，市级项目 1 项。

在过去三十年，因患哮喘所承担的医疗负担，特别是中低收入的国家在过去三十年不断增长。2011 年 9 月，联合国 NCDs 高级别会议正式召开，会议呼吁世界各国关注哮喘带来的日益增大的危害。

二、 突发重大呼吸道传染病的病毒学监控与诊断团队

呼吸道传染病是威胁着人类健康的最主要疾病之一，因其经空气飞沫呼吸道传染科迅速在全球范围内传播，是新发和突发重大传染病的主要内容。近年肆虐的 SARS、H1N1 新型流感、禽流感等重大呼吸道病毒感染不仅严重威胁人类的生命健康，还对国家经济发展、社会稳定带来极大冲击。本团队通过自主研发新型病毒诊断技术，构建病毒监控网络，掌握呼吸道病原学的分布、流行病及临床病变特点，从而指导呼吸道传染病的防控工作。目前团队正在进行 3 型腺病毒表位展示疫苗及载体的研究，今年产出超过 30 个腺病毒的重组体。在临床病毒学监测方面，继续深入开展广东省传染病病原谱流行规律和 COPD 急性加重因素的研究。研究发现，传染病病原谱中各病原体总阳性率 InfA 偏高并普遍存在多重感染的问题，其次出现成人及儿童标本病原体分布差异较大的现象。另外在 COPD 急性加重因素的研究中，

针对 COPD 患者鼻病毒 A/B/C 亚型进行了细致的分析，并展开新亚型全基因组测序工作。研究论文 Detection of Human Bocavirus from Children and Adults with Acute Respiratory Tract Illness in GuangZhou, Southern China.已正式给 *BMC Infectious Diseases* 收录。今年团队运用自主研发的 PCR 技术诊治自疑不明病原感染者，对病毒 EB、HHV6、7 等有了崭新的了解。此外，集资超过 4000 万的实验室产学研基地经过一年的筹建，今年正式举行奠基仪式，计划明年启用。

三、 肺癌的生物学特点，发病机制，治疗、转移机制和预防研究团队

肺癌目前是全世界癌症死因的第一名。1995 年全世界有 60 万人死于肺癌，而且每年人数都在上升，2003 年世界卫生组织(WHO)公布的死亡率是 110 万/年，发病率是 120 万/年。而女性患肺癌的发生率尤其有上升的趋势。该病多在 40 岁以上发病，发病年龄高峰在 60～79 岁之间。男女患病率为 2.3:1。另外种族、家属史与吸烟对肺癌的发病均有影响。

本团队从基因、蛋白质和细胞分子三个层面全面研究肺癌发病机制与开发治疗方法。从基因的角度，本团队选择以 microRNA 做为切入点，发现 microRNA 的异常表达会导致肿瘤的发生、发展、转移，是一种重要的癌症治疗靶点。其中通过环境致癌剂和化学致癌剂诱导的肺细胞恶变中，miR-106a 和 miR-17-5p 在环境致癌方面具有重要作用。而 miR-506 和 miR-542-3p 有抑制化学致癌作用。同时，白藜芦

醇抗肺癌发生过程中，miR-622 呈现抗癌基因作用，可以作为肺癌预防和治疗的潜在靶点等等。相关研究已发表文章在 *American Journal of Respiratory and Critical Care Medicine, Carcinogenesis, Toxicological Sciences* 等国外 SCI 杂志上，取得了重要研究进展。

另外，利用丰富的临床病例资源，建立肺癌肿瘤资料库及抗药性癌株，不但支撑了国内外多家研究机构对肺癌的研究，而且有助于团队对肺癌蛋白质组通道的研究。

从细胞分子水平层面，开发新的合成方法，合成了 100 多种 EGFR 和 Her2 双靶抑制剂，证明化合物 193324 具有抑制激酶不可逆活性并筛选出 4 个具有抗肺癌细胞活性的“苗头”化合物。

在治疗方面，本团队通过对发病机制潜心的研究，总结前人经验，开发出多项新式手术方式应用于肺癌手术。同时引进 CT 和 MR，在术中重建三维图像，使得手术更为精准。结合术中导航设备，引导医生在尽量少损伤正常组织前提下，精确摘除病灶。创新性引进术中放疗，从而扩大微创技术在肺癌治疗中的作用。自主研发廉价微创手术器械，开发出多项实用新型，使得微创手术得以应用于不发达地区，有效降低患者的手术成本。

在癌症预防方面，通过分析疟疾与肺癌的流行病学关系，团队创新性以疟原虫为载体研制治疗性肺癌疫苗，从而奏响人类通过预防征服肺癌的新篇章。

四、 诱发急性肺损伤机理研究团队

对重大呼吸道传染病毒表面蛋白作用呼吸道上皮细胞启动的早期免疫反应信号链进行了深入研究，揭示了 JAK3 激活是病毒抗原诱发免疫病理性炎症反应依赖的关键节点。应用 JAK3 敲除小鼠与短路电流技术初步揭示了禽流感病毒表面膜抗原 HA 通过激活呼吸上皮细胞 JAK3 抑制了 AC 使 cAMP 依赖的 CFTR－氯离子通道开放受阻；进而激活 NF-κb 信号转导通路，加剧病理性免疫炎症反应；免疫炎症能够干扰机体免疫系统，增强对定植在上呼吸道条件致病菌细菌毒素的炎症反应。选择性抑制 JAK3 能够显著减轻病毒及其表面蛋白诱导呼吸上皮异常固有免疫应答导致的肺炎及免疫器官的损伤。研究发现：持续感染 RSV 病毒的气道上皮细胞能显著增加 T 细胞增殖，促进淋巴细胞的凋亡，并且提高 IFNγ、IL-4 及 IL-17 的分泌水平。初步揭示 HBECs 持续感染 RSV 能使 Th 细胞亚群异常漂移，诱导肺内免疫失衡与异常激活，导致免疫病理性炎症的发生发展。对血管内皮衍生微粒（endothelium-derived microparticles, EMP) 释放在急性肺血管损伤中的作用研究发现：病理浓度 EMP 能抑制新生血管形成，通过激活 JAK/STAT

信号通路介导的免疫炎症反应，可能参与了急性肺损伤早期的血管内皮毛细血管破坏。

研究了内源性损伤因子 DAMPs 的代表 HMGB1 激活 AKT 对 STAT3/p63/Jagged/Notch 信号转导的交互联接，多位点整合调节宿主免疫病理应答与 EMT 发生的机制。结果显示：HMGB1 能刺激肺泡

上皮细胞 Akt 信号活化，提示释放到细胞外的 HMGB1 参与了免疫炎症环境中 EMT 的发生。利用成功构建的条件性敲除 HMGB1 小鼠，我们证实 HMGB1 参与了博莱霉素介导的肺纤维化，这些提示：HMGB1 作为一种“DAMPs”在肺内的持续性存在是促发与维持异常纤维增生的重要危险因子。

我们探讨了人骨髓间充质干细胞（MSC）的免疫调节抗炎及其保护肺上皮细胞的抗损伤机制，调查了 MSC 对 NSIP 患者肺成纤维细胞炎症表型与分化的影响。研究发现 MSC 通过提高免疫炎症损伤局部 TGF-β1 的水平发挥其对免疫细胞亚群的调节作用（诱导调节性 T 亚群增殖与分化），继而产生抗炎、抗损伤效应，与此同时刺激 IP-10 的表达有助于拮抗过量 TGF-β1 对成纤维细胞的活化作用，从而诱导肺泡上皮细胞的再生与功能分化，这是间充质干细胞在肺间质炎症纤维化发生发展过程中有可能发挥抗炎抗纤维化治疗作用的重要基础。

五、 慢性阻塞性肺疾病发病机制与防治研究团队

从 2001 年慢性阻塞性肺疾病全球倡议公布至今，慢性阻塞性肺疾病(以下简称 COPD)已日益引起世界各国研究单位的关注。但是，COPD 的危害性还未能引起世界各国政府的足够关注，以致相关公共卫生预防措施还未得到广泛的开展，公众对其认识不足。

COPD 是美国第四大致死性疾病，在中国位于第三位。在中国，COPD40 岁以上人群的总体患病率为 8.2%，男性为 12.4%，女性为

5.1%。数据显示 COPD 的发病率致死率仍然呈上升趋势。本团队根据 COPD 的病理特征与临床表现对其发病因素及机制、气道粘膜损伤、肺动脉高压、呼吸困难的机制以及防治和外周骨骼肌消耗评价进行了系统研究，从而指导 COPD 的早期筛查，提高其综合防治技术。

团队今年取得三项科技进步奖。“γ-谷氨酰半胱氨酸合成酶在 COPD 发病中的作用与机制研究”获得广东省科技进步二等奖和广州市科技进步一等奖；“TRPC 蛋白在低氧性肺动脉高压发病中的作用与机制研究”获得广州市科技进步二等奖(登记号 GK11036）。在 COPD 肺动脉高压发病机制的研究中，团队创新性地引入丹参酮 IIA 干预肺动脉高压。研究发现，丹参酮ⅡA 能有效降低低氧性肺动脉高压大鼠右心室压力，相关机制还在探索中。另外在 COPD 外周骨骼肌消耗机制和评价研究方面，团队发现血清反应因子(SPF)在 COPD 气道肺血管重塑和骨骼肌萎缩中有重要干预作用。

目前，业界对 COPD 的发病机制还在摸索中，但是对 COPD 进行早期筛选能有效降低 COPD 的发病率却是业界的共识。团队通过对肺功能正常值的研究、COPD 简易筛查技术、COPD 生物标志物探索、COPD 患者早期药物治疗效果观察及药物基因组学的研究，开发适宜基层社区 COPD 初筛和综合防治技术。今年团队的全国肺功能正常值的研究已进入尾声，明年全国肺功能正常值将正式出台，这对开展 COPD 早期筛查有重要的指导性意义。倡导环境保护，减少生物燃料及烟雾污染；建立社区早期筛查模式，构建符合国情的 COPD 预防与规范诊治体系将是团队十二五计划的目标之一。

第五部分：呼吸疾病国家重点实验室学术委员会会议纪要

会议时间：2011 年 12 月 16 日(星期五)14:30~17:30

参会人员：

- 学术委员会委员：钟南山、刘又宁、王辰、白春学、裴端卿、王小宁、陈凌、陈小平、陈荣昌、徐军
- 实验室 PI：何建行、黎毅敏、莫自耀、赖克方、陶爱林、卢文菊、郑劲平、黄庆晖、孙宝清等。

会议记录及整理：黎明、黄晓亮

会议内容：

会议由学术委员会副主任刘又宁教授主持。实验室主任钟南山院士首先向学术委员会汇报了实验室 2011 年的总体发展情况，分别介绍新增项目、获得科研经费资助、发表文章数、SCI 文章数、专利及成果，以及人才培养和学术交流等方面所取得的成绩，同时提出了实验室发展目前存在的问题和改进措施。紧接着实验室 PI 徐军、卢文菊、赖克方和何建行分别就肺损伤和肺感染、慢性阻塞性肺疾病(COPD)、慢性咳嗽和哮喘以及肺癌四个研究学组所取得的主要研究进展分别进行了详细的工作汇报。随后，各学术委员对实验室的发展情况提出了许多建设性意见，主要归纳如下：

1. 实验室在过去的一年取得了明显的进步；
2. 实验室应继续坚持基础与临床结合，保持和发挥自己的优势，引领国家呼吸疾病的防控；
3. 实验室应该更加突出重点研究方向，明确重点研究内容，加大人力、物力等投入，争取高水平的研究成果和论文；
4. 实验室应加强规划，明确工作目标；
5. 实验室应加强更高水平的人才引进与培养。

附：会议交流发言记录：

(一) 王小宁：

1. 跟上次中期评估对比，这次的汇报让人耳目一新，实验室的发展分为四个方向，发展方向更加明确清晰。
2. 国家重点实验室是以基础研究为出发点，但应着重于临床应用与转化，在国内呼吸疾病研究中发挥引领作用。目前呼吸道疾病如 COPD 在国内的诊治仍面临许多困难，如公众和社会对疾病的严重危害认识不足。
3. 今年实验室取得的成绩显著。论文数量和质量明显上升。SCI 大于 10 的有 2 篇。但重要的学术论文如 CNS 有待加强。
4. 在人才的培养及引进方面，广州的人才聚集氛围明显不如北京、上海和中科院体系。可能受广州的商业文化背景影响。
5. 实验室增加了三个联盟(如新引进了陶爱林、谭守勇等 PI)，让重点实验室的构架更加丰满。但要考虑引进更高层次的人才，实验室应该加强申请和招募。
6. 四个课题组的汇报都超时了。根本原因在于各方向较散，集中度不够。可考虑将研究内容划出层次，强调亮点(如 COPD 以及实验室在转化医学、社区干预以及学科带头方面的作用)。科技部的评估条件还有转变的可能，实验室应更看重自身对社会的贡献。而不仅仅体现在 SCI 文章数量和点数上。
7. 研究肺癌的实验室国内有不少，呼吸疾病国家重点实验室应该突出自身的特点。考虑以肺癌种类和吸烟方面为主。
8. 故此我的建议主要总结为两点：①调整合理研究方向；②突出各团队研究重点。

(二) 王辰

1. 这次汇报体现实验室的进步是显著的；
2. 研究系统性更强，每个团队的研究内容和工作开展更为全面；
3. 凝练研究结构主线，将人力、物力主要投入在研究的亮点上面；
4. 目前实验室在肺损伤、呼吸衰竭和呼吸危重症方面已经做了大量的工作

了。实验室是否考虑加强肺损伤和呼吸衰竭方面的研究和临床转化，并将呼吸与危重医学捆绑发展，并引领此模式推向全国，从而推动呼吸危重症的发展，推动新呼吸系统研究格局的形成。

5. 在基础研究上面多做工作，加强与临床结合。实验室作为一个临床转化实验室，加强危重症的研究对学科的发展甚至社会的发展都具有十分重大的意义。

(三) 裴端卿

1. 高端人才的引进可以通过合作来实现。今年健研院又引进了一名人才，可以加强呼吸实验室的力量。目前健研院与呼研所两个单位的PI 的融合已经做得很好了。今后彼此的合作，可以通过加强行政架构的整合，同时深入细化双方的研究内容，争创更高水平的成果。
2. 实验室在产业化方面的能力有待加强。健研院在药物研发方面的经验很足，并且已经初具规模。实验室可以考虑联合健研院的研发体系，共享成果。
3. 当前国家对实验室的评估主要落在 SCI 点数上面。但是我估计今后的发展，还是会考察实验室是否对国家社会的发展做出突出贡献的。所以建议实验室在解决临床问题上面多下功夫，争取在临床上出大成果。

(四) 白春学

1. 实验室目前研究点太多，重点不突出。建议每个方向凝炼成一两点，不宜过于分散；建议集中全实验室的资源去做好一个亮点。
2. 明确方向，不知道实验室各课题组交叉融合如何？
3. 现在学术界越来越重视“炎症与肿瘤”的关系，实验室是否加大在这点的投入力度；

(五) 陈凌

1. 目前实验室还是以 PI 自由探索为主，缺少规划。我相信过几年高端人才的引进，高水平 SCI 的产出还是十分必要的。
2. 明年的目标不明确；
3. 管理和计划要规范化，如集中研究目标和精力，争取出几篇 CNS 或重大成果；

4. 目前实验室明确为四个学组方向。建议更突出重点，投入力度将一个亮点做到世界级水平。

(六) 徐军

1. 经费运用效率较低，建议凝练目标提高效率。如 973 项目是重大项目，但人力不足；建议围绕重点项目，加大对其投入力度；
2. 建议实验室不应过分强调文章的数量，更应该注重学术的质量。过分强调文章数量，有可能会造成急功近利的心态，不利于实验室的长期发展。

(七) 陈荣昌

1. 实验室今年获南粤功勋奖，每年还有国家、省、地方配套以及依托单位的支持等等。其实我们是拥有较好的研究平台的。今后会加强对学术研究的支持力度。

(八) 陈小平

1. 学术委员会的点评很到位。建议室务会凝练目标；
2. 建议实验室在政策层面引导 PI 确立目标；
3. 根据凝练的目标，调整科研经费对项目的支持。通过机制、经费保证重点项目的开展。

(九) 刘又宁

1. 实验室已经取得很不错的成绩了。基础研究带动临床，效益很高。目前的问题只是如何做的更好，拿出更高水平的成果。建议通过国际的横向对比确定重点；
2. 产学研结合很好，但是有些优势不能丢。比如：疟原虫、COPD 和肺功能正常值的研究。目前实验室在 COPD 方面的研究已经是国内第一，明确了国人 40 岁以上 COPD 的发病率，有可能是烟雾或者空气污染导致 COPD(有别于西方认为的没有吸烟就没有 COPD 的观点)。还有肺功能正常值的出台。但是最重要的是如何结合两者提出 COPD 的早期干预的措施；
3. 目前实验室在肺功能、机械通气和呼吸生理三方面已经做得很好了。随着现在慢性咳嗽发病率的增大，其临床应用意义还是巨大的。这些都是优势。现在实验室面临的问题就是如何高效运用经费，突出研究重点。在人才培养方面，通过与国内其他呼吸研究所合作，扩大自身的辐射影

响作用。

(十) 卢文菊

考虑关注小儿呼吸道疾病。

(十一) 白春学

小儿呼吸道疾病的确是很不错的一个发展方向。正如前面我们谈到的，要集中精力做好几样。如果实验室这方面基础做的好，可以加大投入；但是如果基础一般，实验室就要考虑是否有足够的资源将其发展成为具有标志性的成果了。

（十二）钟南山

1. 因为这次的汇报大会的准备时间较仓促，四个方向之间来不及详细交流。所以未明确下一步的计划。接下来实验室会召开一次会议，集思广益，明确明年的目标方向。重点还会落在临床应用与基础研究上面。而基础研究的创新性将会是实验室重点扶持的；
2. 每次实验室学术委员会会议，最主要还是要通过交流和听取委员们的意见合建议，确定实验室的研究方向。当然也会谈谈管理的规范化。大家对汇报超时的根本原因总结的十分准确，就是本身研究方向的凝练不足。当然部分原因也要归咎于我们事前准备工作的不足。；
3. 实验室起步初期，我们都是鼓励大家先自由选题。做出一定成绩后，申报国家项目，造成了研究方向不明确。现在实验室的发展已经上了轨道了。我们会考虑如何通过不同项目之间的有机整合，明确实验室今后发展的大方向；
4. 我个人一直在平衡资源整合和大家的科研积极性的问题。理清头绪之后实验室就会调整战略。刘教授刚刚提到的三点，COPD、呼吸生理、慢性咳嗽十分正确，问题是实验室如何有意识地从这三方面去规划发展；
5. 目前实验室的发展主线是临床应用加上基础研究，但是问题是如何从架构上面去体现。今年实验室引进了多伦多大学的张海波，张教授是呼吸危重症方面的专家，也涉及部分基础研究。目前实验室思考的是如何有机整合基础、临床、产学研和呼吸危重症等的研究工作；
6. 因为重点实验室的依托单位广州医学院并非国家 211 工程的大学，所以

依托单位对实验室的支持有限，导致实验室在人才引进方面有所局限。刚刚提到健研院今年又引进一位人才。主要是从事癌症T细胞受体方面研究的。呼研所会加强与健研院的有机结合，同时加强自身人才队伍的培养，真正做到人才强室，有序发展。

第六部分：政府及依托单位给予的支持

在呼吸疾病国家重点实验室的长期发展中，省市领导及依托单位广州医学院都给予了极大的帮助和支持。在今年 3 月份的实验室评估工作中，广州医学院的领导高度重视，由冉丕鑫书记、张雅洁副校长、王健处长等组成的考察专家小组多次听取了实验室的工作汇报，并对实验室建设提出了宝贵的建议，为实验室评估获优铺垫了良好的基础。

自 2008 年起广东省教育厅和广州市财政局分别按每年 50 万元和 200 万元预算，共 250 万元的额度作为实验室日常运行经费，这是保障重点实验室正常运转的重要基础。除此之外，本年度实验室还获得了科技部 760 万的重点实验室建设经费、教育部 350 万特色重点专科经费以及省政府拨付 2500 万元的资助，这都将更好地支撑实验室引进国内外精尖人才、购置先进仪器设备、培养骨干人才等。

与此同时，正在筹建中的广州呼吸中心也得到了省市领导的高度关注。省市共建广州呼吸中心的设想在 2009 年提出之时，得到了汪洋书记的充分肯定，并批示由省政府牵头推进相关工作，朱小丹省长随即主持召开了市委办公会，明确指示“将这项建设列为广州社会事业建设的重点工程，立足广州，创新行政管理体制，实现资源优化组合，共同服务好全省、全国。”

第七部分：依托单位年度考核

关于 2011 年度呼吸疾病国家重点实验室年度考核意见：

2011 年呼吸疾病国家重点实验室在管理方面进一步完善了管理制度，制订了《呼吸疾病国家重点实验室管理规章制度》、《国家重点实验室外聘 PI（课题负责人）制度》等重要文件，实验室按期举行了学术委员会会议，进一步凝练了实验室研究方向。

本年度实验室在科学研究方面取得良好成绩，获得省部课题 47 项，获得科研经费 5418.1 万元，较去年增长了 38.7%，其中国家自然基金 24 项；实验室共发表 SCI 论文 144 篇，影响因子大于 20 的 SCI 论文有 1 篇，大于或等于 10 的有 3 篇，大于或等于 5 的有 13 篇，大于或等于 3 的有 39 篇，平均影响因子为 3.4898 ；本年度实验室获授权的发明专利共 1 项，实用新型专利 7 项，申请专利共 22 项；获得科研奖项 11 项，其中 2 项教育部，1 项省级，4 项市级奖项。

在人才培养方面，实验室引进广州医学院第二附属医院陶爱林教授、胸科医院谭守勇教授及加拿大多伦多大学张海波教授作为实验室 PI 参与实验室的科学研究；同年培养博硕士 27 名。

通过年度考核。

广州医科大学

2012 年 2 月 21 日

第八部分：附录

一、 2011 年呼研所暨国家重点实验室大事记

2 月份

2 月 15 日钟南山在广东省博物馆“南都健康大讲堂”讲学

2 月 17 日重点实验室迎评动员和“实验室管理规章制度”培训会

2 月 24 日沈阳市胸科医院来呼研所参观

3 月份

3 月 2 日下午国家重点实验室评估会议

3 月 3 日下午沈阳市胸科医院来呼研所参观交流

3 月 17 日特异性免疫治疗百周年研讨会巡回演讲（李靖）

3 月 25 日国家科技部社会发展司杨哲司长莅临实验室调研

3 月 25 日 SFDA 来我院及呼研所考察临床药物试验机构

3 月 25 日香港黄大仙医院来所进行学术交流活动

3 月 21 日至 3 月 27 日呼吸和危重症监护医学学习班/呼吸系统疾病诊疗技术新进展学习班

3 月 25 日至 3 月 27 日第二届广州（国际）COPD 和睡眠大会

4 月份

4 月 10 日张海波教授来访探讨中加联合实验室规划建设方案

4 月 11 日呼研所青年人才培养座谈会

4 月 22 日钟院士及呼研所专家一行 5 人莅临广东出入境检验检疫局参加聘请钟南山院士为广东局学术顾问聘书及“国家生物安全检测重点实验室-呼吸疾病国家重点实验室联合 BSL-3 实验室”揭牌仪式

4 月 29 日省财厅、省教育厅来我校调研，主题“促进教育优先发展的财政政策”，同时，省财厅沈梅红副厅、省教育厅罗远芳巡视员等参观重点实验室

5 月份

5 月 5 日哮喘日义诊暨重点实验室公共开放日

5 月 6 日不明原因病毒专家研讨会及记者招待会

5 月 12 日“2011 穗台医疗高峰论坛”台湾嘉宾莅临我院及呼研所参观交流

5 月 27 日-28 日第五届全国不明原因慢性咳嗽暨疑难少见病诊断与治疗学习班

6 月份

6 月 16 日日本富山大学 Katsuhiro Konno 教授和澳门大学 Simon Lee 教授访问呼吸疾病国家重点实验室并进行学术交流及青年培养模式研讨会

6 月 25 日至 6 月 26 日亚太肺癌论坛和肺叶切除大汇演

7 月份

7 月 4 日呼吸疾病国家重点实验室青年基金答辩会

7 月 8 日第七个世界过敏日大型教育宣讲及义诊活动，主题为“摆脱过敏，自在生活”。

7 月 29 日华南新药创新中心产学研项目研讨会

8 月份

8 月 4 日国家自然科学基金二审评审体会座谈会。由张海波教授主讲，

刘宇平副院长主持。

8 月 6 日-8 日国际肺损伤学术会议（徐军），会议以在研 973 项目研究的核心内容，“肺发育、损伤修复和再生”为主题。

8 月 17 日咳嗽组邀请专家 PETER 来访

8 月 23 日板蓝根抗流感病毒感染的有效成分（群）研究进展汇报暨产学研研讨会

8 月 24 日比尔及梅琳达.盖茨基金会参观国家重点实验室

8 月 25 日 COPD 患者座谈会，主题为“倾听您的声音”

8 月 26 日-28 日支纤镜学习班（李时悦）

9 月份

9 月 2 日-4 日儿童呼吸学习班（陈爱欢）

9 月 15 日-18 日第十二次全国呼吸病学术会议

9 月 19 日美国加州大学孙兴国教授来呼研所肺功能室参观讲学

10 月份

10 月 12 日召开研究骨干动员会议，布置国家自然科学基金课题的申报

10 月 13 日美国加州儿童肺功能专家 David 来我所参观讲学

10 月 26 日“阿斯利康：名院名家面对面”学术交流会议

11 月份

11 月 3 日省发改委来呼研所考察省工程实验室项目

11 月 8 日-12 日全国肺功能学习班（郑劲平、高怡）

11 月 16 日淋巴管肌瘤专病门诊挂牌仪式

11 月 16 日香港中文大学陈小章教授作学术报告《From epithelial cells to host defense of the lung》

11 月 18 日贝尔.盖茨基金会及广州生物岛科技公司到访实验室

11 月 23 日 COPD 课题组年终总结汇报

11 月 24 日 COPD 日大型义诊活动暨社区医生培训

11 月 26 日咳嗽哮喘课题组年终总结汇报

11 月 28 日肺感染和肺损伤课题组总结汇报

11 月 30 日肺癌课题组总结汇报

12 月份

12 月 5 日 PI 年度工作总结大会

12 月 7 日尼泊尔医学会来重点实验室参观交流

12 月 16 日实验室学术委员会会议

二、 发表论文首页

通讯作者类论文

SCIENCE CHINA
Chemistry

• ARTICLES •
• SPECIAL TOPIC • In Honor of the 80th Birthday of Professor Ronald Breslow

November 2011 Vol.54 No.11: 1702–1710
doi: 10.1007/s11426-011-4398-4

5-Ethynyl-2′-deoxyuridine as a molecular probe of cell proliferation for high-content siRNA screening assay by "click" chemistry‡

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Received July 31, 2011; accepted August 16, 2011; published online October 11, 2011

Labelling and identification of proliferating cells is important for the study of physiological or pathological processes in high-content screening (HCS) assays. Here we describe ethynyl deoxyuridine (EdU) as a biomarker for the assessment of cell proliferation and clearly demonstrate the feasibility of the EdU-labelling method for use in HCS assays. EdU detection is highly robust, reproducible, technically simple, and well suited for automated segmentation, which provides an excellent alternative for setting up multiplexed HCS assays of siRNA, miRNA and small-molecule libraries.

molecular probe, siRNA, miRNA, "click" chemistry

1 Introduction

High-content screening (HCS) is defined as multiplexed functional screening based on imaging multiple targets in the physiologic context of intact cells by extraction of multicolour fluorescence information [1], which has played a key role in cell proliferation and functional studies. Labelling, detection or quantification of either proliferating cells or cells in the S-phase of cell cycle progression is important in order to study physiological or pathological processes involved in cell proliferation using HCS. Recently, 5-ethynyl-2′-deoxyuridine (EdU), a new, non-radioactive thymidine analogue, was developed by replacing the methyl group at the 5-position of thymidine with an alkynyl group and was shown to readily incorporate into DNA during cell proliferation [2, 3]. The terminal alkynyl group of EdU can react with fluorescent azide in a Cu(I)-catalysed [3+2] cycloaddition, known as a "click" reaction, enabling detection of EdU incorporation into cells by fluorescence microscopy or fluorescence-activated cell sorting. Few groups have reported the use of EdU in HCS. In this study, we investigated the application of EdU in multiplexed HCS assays.

2 Materials and method

2.1 Chemicals

EdU was synthesized from 5-iodo-deoxycytidine and trimethyl-silylacetylene by the Sonogashira coupling reaction (Figure 1(b)), as previously described [4]. The identity and purity of EdU was confirmed by TLC, NMR, and mass spectrometric analysis. EdU, dissolved in DMSO at 100 mM, was stored frozen. The fluorescent azide derivatives Cy3-azide

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†Equal contribution to this work.
‡Dedicated to Prof. Ronald Breslow on the occasion of his 80th birthday

Analytical Biochemistry 417 (2011) 112–121

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

5-Ethynyl-2′-deoxycytidine as a new agent for DNA labeling: Detection of proliferating cells

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ARTICLE INFO

Article history:
Received 30 March 2011
Received in revised form 19 May 2011
Accepted 23 May 2011
Available online 30 May 2011

Keywords:
"Click" chemistry
EdU
5-Ethynyl-2′-deoxycytidine
DNA labeling
Cell proliferation
dTMP synthetase

ABSTRACT

The labeling of newly synthesized DNA in cells to identify cell proliferation is an important experimental technique. The most accurate methods incorporate [^{3}H]thymidine or 5-bromo-2′-deoxyruidine (BrdU) into dividing cells during S phase, which is subsequently detected by autoradiography or immunohistochemistry, directly measuring the newly synthesized DNA. Recently, a novel method was developed to detect DNA synthesis in proliferating cells based on a novel thymidine analog, 5-ethynyl-2′-deoxyuridine (EdU). EdU is incorporated into DNA and subsequently detected with a fluorescent azide via "click" chemistry. This novel technique is highly sensitive and does not require DNA denaturation. However, it was also found that EdU exhibits time-dependent inhibition effects on cell growth. Therefore, here we report a novel deoxycytidine analog, 5-ethynyl-2′-deoxycytidine (EdC), that can be used to detect DNA synthesis in vitro and in vivo at a similar sensitivity level compared with EdU. Furthermore, the EdC-induced cytotoxicity is much less than that of EdU when combined with thymidine. This will be a potential application for the long-term detection of proliferating cells.

The detection of proliferating cells is a fundamental experimental tool for assaying basic biology, assessing cell health, and evaluating the cytotoxicity of lead compounds. The traditional method of detecting cell proliferation is the incorporation of a thymidine analog, [^{3}H]thymidine or 5-bromo-2′-deoxyruidine (BrdU),[1] into duplicated DNA during S phase. Although the classical method of [^{3}H]thymidine labeling is very powerful, it exhibits several drawbacks [1]. During the mid-1980s, a method that uses a nonradioactive analog of thymidine, BrdU, to replace [^{3}H]thymidine was developed. Similar to [^{3}H]thymidine, BrdU can be incorporated into duplicate DNA and subsequently detected with a BrdU-specific antibody to label cell proliferation [2]. Although this BrdU method has gained popularity due to its benefits, there are also a few significant limitations to its overall application. Because the size of the monoclonal antibody is too large to approach the BrdU molecules that are masked within the DNA, this method requires a harsh DNA denaturation step to reveal the epitope for the binding of the anti-BrdU antibody. The denaturation procedure typically includes treatment with hydrochloric acid or heating. Thus, these strong denaturation conditions may destroy tissue structure and other protein epitopes of interest within the tissue and hinder the multiplex analysis of this classical antibody staining technique.

Recently, a novel thymidine analog, 5-ethynyl-2′-deoxyuridine (EdU), was developed as an alternative to [^{3}H]thymidine or BrdU for the labeling of DNA in proliferating cells [3,4]. The terminal alkyne group of EdU can be detected with fluorescent azides via "click" chemistry [5–7]. Because the size of the fluorescent azide reagents is much smaller than that of their corresponding antibodies, the fluorescent azides can easily diffuse through tissues and approach the incorporated EdU without DNA denaturation. The EdU incorporation method has been successfully employed in the detection of cell proliferation in the nervous system [8,9], chick embryos

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[1] *Abbreviations used:* BrdU, 5-bromo-2′-deoxyruidine; EdU, 5-ethynyl-2′-deoxyuridine; EdC, 5-ethynyl-2′-deoxycytidine; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DAPI, 4′,6-diamidino-2-phenylindole; NMR, nuclear magnetic resonance; Cy3-azide, 2-[3-(1,3-dihydro-1,1-dimethyl-3-(6-azidohexyl)-2H-benz[*e*]indol-2-ylidene) propenyl]-3,3-dimethyl-1-ethyl-3H-indolium bromide; DCM, dichloromethane; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; HCS, high-content screening; i.p., intraperitoneally; dCK, deoxycytidine kinase; EdUMP, 5-ethynyl-dUMP.

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doi:10.1016/j.ab.2011.05.037

Food Chemistry 129 (2011) 541–545

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

A two-site monoclonal antibody immunochromatography assay for rapid detection of peanut allergen Ara h1 in Chinese imported and exported foods

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ARTICLE INFO

Article history:
Received 2 November 2010
Received in revised form 22 February 2011
Accepted 19 April 2011
Available online 30 April 2011

Keywords:
Peanut allergen
Ara h1
GICA
Food allergen detection

ABSTRACT

Food allergen labeling has not yet been implemented in China. Therefore, a gold immunochromatography assay (GICA) was developed using two monoclonal antibodies (mAb) against the peanut allergen Ara h1. The GICA was specific for standard peanut samples with a sensitivity of 10 ng/ml. Peanut protein traces extracted from 124 food products imported and exported by China Customs were easily and rapidly detected by GICA. 68 food samples originally labeled as containing peanuts were positive for Ara h1 and 54 food samples labeled as not containing peanuts were negative for Ara h1, indicating that the labels from the manufacturers were accurate. However, 2 food samples labeled as not containing peanuts tested positive for Ara h1. The present GICA provides a fast, simple, semi-quantitative method for the determination of peanut allergens in foods. This detection system can be used to ensure the safety of food imported and exported by China Customs.

1. Introduction

Food containing peanut ingredients can be allergenic and may even cause life-threatening allergic reactions (Bock, Munoz-Furlong, & Sampson, 2001, 2007). Death resulting from peanut allergies has been reported both in China and the USA (Ji et al., 2009). Threshold doses for allergic reactions can be as low as 100 μg of peanut protein (Hourihane et al., 1997). As there is currently no effective causative therapy for peanut allergies, labeling of food containing peanut ingredients has proven an effective and simple method for avoiding allergic reactions (Vierk, Koehler, Fein, & Street, 2007). The Food and Drug Administration (FDA) Bureau of the USA implemented a regulation named Food Allergen Labeling and Consumer Protection Act (FALCPA) in Jan 2006, requiring food allergen labeling of all foods sold in the USA (FDA, 2004). Similar regulations have been passed in other developed regions, such as Japan and the European Union (Li et al., 2009). However, no such regulation has been implemented in China, although food import and export between these developed countries and China is increasing (McKay, 2007). Therefore, it would be beneficial for Chinese Customs to be able to perform rapid detection of peanut allergens in order to establish a food safety surveillance system and avoid international trade disputes.

Ara h1 is an ideal biomaker for peanut allergen detection for a number of reasons (Pomés et al., 2003). Firstly, Ara h1 is the major component of peanut, comprising 12–16% of the total peanut protein (Koppelman et al., 2001). Secondly, it has a stable structure so that it does not degrade during food processing procedures, such as heating or roasting (Beyer et al., 2001). Thirdly, Ara h1 is the most important major allergen from peanuts.

Food detection systems used by Customs should be fast, sensitive and specific. GICA has been commonly used previously by Customs because it is quick and easy to use (Stephan, Möller, Lehmann, Holzhauser, & Vieths, 2002). Lateral flow devices based on polyclonal antibodies for the detection of peanut allergens have previously been shown to be simple, sensitive and rapid (Wen, Borejsza-Wysocki, DeCory, & Durst, 2005). However, false positive results can be obtained with this approach due to cross-reactivity of the polyclonal antibodies (Schubert-Ullrich et al., 2009). The high specificity of mAbs can be used to address the problem of false positives. In this study, we developed a new, two-site Ara h1 specific, mAb-based sandwich GICA method which allows the rapid, highly selective and simple semi-quantitative determination of Ara h1 from food products imported and exported by China Customs.

2. Materials and methods

2.1. Purification and identification of Ara h1

Fresh peanuts were supplied by a local vendor. Ara h1 was purified from peanut extract by ammonium sulfate precipitation and ion exchange chromatography as previously reported (Maleki et al., 2000). The purified native Ara h1 (nAra h1) was separated

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doi:10.1016/j.foodchem.2011.04.055

OPEN ACCESS Freely available online

PLoS one

Antitumor Effect of Malaria Parasite Infection in a Murine Lewis Lung Cancer Model through Induction of Innate and Adaptive Immunity

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Abstract

Background: Lung cancer is the most common malignancy in humans and its high fatality means that no effective treatment is available. Developing new therapeutic strategies for lung cancer is urgently needed. Malaria has been reported to stimulate host immune responses, which are believed to be efficacious for combating some clinical cancers. This study is aimed to provide evidence that malaria parasite infection is therapeutic for lung cancer.

Methodology/Principal Findings: Antitumor effect of malaria infection was examined in both subcutaneously and intravenously implanted murine Lewis lung cancer (LLC) model. The results showed that malaria infection inhibited LLC growth and metastasis and prolonged the survival of tumor-bearing mice. Histological analysis of tumors from mice infected with malaria revealed that angiogenesis was inhibited, which correlated with increased terminal deoxynucleotidyl transferase-mediated (TUNEL) staining and decreased Ki-67 expression in tumors. Through natural killer (NK) cell cytotoxicity activity, cytokine assays, enzyme-linked immunospot assay, lymphocyte proliferation, and flow cytometry, we demonstrated that malaria infection provided anti-tumor effects by inducing both a potent anti-tumor innate immune response, including the secretion of IFN-γ and TNF-α and the activation of NK cells as well as adaptive anti-tumor immunity with increasing tumor-specific T-cell proliferation and cytolytic activity of $CD8^+$ T cells. Notably, tumor-bearing mice infected with the parasite developed long-lasting and effective tumor-specific immunity. Consequently, we found that malaria parasite infection could enhance the immune response of lung cancer DNA vaccine pcDNA3.1-hMUC1 and the combination produced a synergistic antitumor effect.

Conclusions/Significance: Malaria infection significantly suppresses LLC growth via induction of innate and adaptive antitumor responses in a mouse model. These data suggest that the malaria parasite may provide a novel strategy or therapeutic vaccine vector for anti-lung cancer immune-based therapy.

Citation: Chen L, He Z, Qin L, Li Q, Shi X, et al. (2011) Antitumor Effect of Malaria Parasite Infection in a Murine Lewis Lung Cancer Model through Induction of Innate and Adaptive Immunity. PLoS ONE 6(9): e24407. doi:10.1371/journal.pone.0024407

Editor: Hong Wei Chu, National Jewish Health, United States of America

Received May 18, 2011; **Accepted** August 8, 2011; **Published** September 9, 2011

Funding: Grant support from the National Natural Sciences Foundation of China (No. 81001022 to ZH), a grant from the State Key Laboratory of Respiratory Disease to XC, and a grant from the CAS-FS Biotech and Pharmaceutical Center to XC. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. Although treatment methods in surgery, irradiation, and chemotherapy have been improved, prognosis remains unsatisfactory, and developing new therapeutic strategies is still an urgent demand. Immunotherapy may represent one of new therapeutic strategies for lung cancer has recently been developed [2–5]. The goal of lung cancer immunotherapy is to augment the weakened host immune response against tumors using specific and/or nonspecific immune stimulants [4–6]. Nonspecific immunostimulatory agents and interventions with cytokines have limited clinical benefits. The target-directed immunotherapy with defined tumor antigens, such as melanoma-associated antigen 3 and mucin 1(MUC1), are suboptimal and strong adjuvant agents are needed [6,7]. In addition, it is now clear that lung cancer often present a tolerogenic microenvironment that hampers effective antitumor immunity. Therefore, new potent and efficacious immunotherapy, both augmenting antitumor immunity and counteracting tumor-mediated immunosuppression for lung cancer are needed.

Malaria, which is caused by an intracellular parasite from the *Plasmodium* genus, is the most common parasitic infection in humans. Human malaria parasite infection can produce periodic high fevers in the acute phase. Hyperthermia has been clinically used for the treatment of certain cancers [8–11]. Furthermore, malaria has been reported to stimulate host immune responses, such as promoting IFN-γ production, activating natural killer (NK)

Carcinogenesis vol.0 no.0 pp.1–10, 2011
doi:10.1093/carcin/bgr272
Advance Access publication November 22, 2011

A functional polymorphism at microRNA-629-binding site in the 3′-untranslated region of *NBS1* gene confers an increased risk of lung cancer in Southern and Eastern Chinese population

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The genetic variations in *NBS1* gene have been reported to be associated with cancer risk. The polymorphisms in 3′-untranslated region (3′-UTR) of *NBS1* might affect gene's function and thus contribute to cancer susceptibility. We hypothesized that these polymorphisms of *NBS1* are associated with the lung cancer risk. In two independent case–control studies conducted in Southern and Eastern Chinese, we genotyped three tagSNPs (rs14448, rs13312986 and rs2735383) in Southern Chinese and then validated the discovered association in Eastern Chinese. No significant association was observed for rs13312986 and rs14448; we only found that the rs2735383CC genotype had a significantly increased risk of lung cancer under a recessive genetic model in the total 1559 cases versus 1679 controls (odds ratio = 1.40, 95% confidence interval = 1.18–1.66, $P = 0.0001$) when compared with GG or GC genotypes; the rs2735383CC genotype carriers had lower messenger RNA and protein expression levels in tumor tissues than those of other genotypes as quantitative polymerase chain reaction and western blot shown. Luciferase assay revealed that the rs2735383C allele had a lower transcription activity than G allele, and the hsa-miR-629 but not hsa-miR-499-5P had effect on modulation of *NBS1* gene *in vitro*. We further observed that the X-ray radiation induced more chromatid breaks in lymphocyte cells from the carriers of rs2735383CC homozygote than those from the subjects with other genotypes ($P = 0.0008$). Our data suggested that the rs2735383G>C variation contributes to an increased risk of lung cancer by diminishing gene's expression through binding of microRNA-629 to the polymorphic site in the 3′-UTR of *NBS1* gene.

Introduction

DNA double-strand breaks (DSBs) are extremely serious DNA damage, if unrepaired, it will cause genomic instability and thus cancer susceptibility (1–3). Carcinogens and exposure to ionizing radiation, as well as cellular metabolism during replication, meiosis and V (D) J recombination, often cause abundant DSBs in human cells (4–9), while two independent DNA repair pathways, the homologous recombination (10) and non-homologous end-joining response, will recruit a series of DNA repair-related protein to the site of DSBs and repair them (11,12). Many molecules have been identified involved in DNA repair, such as BRCA1, BRCA2, XRCC1, Mre11, RAD50 and NBS1, and somatic mutations or genetic variants in these genes were associated with the risk of various cancers including lung cancer (13–18).

Abbreviations: CI, confidence interval; DSB, double-strand breaks; htSNP, haplotype-tagging SNPs; LD, linkage disequilibrium; mRNA, messenger RNA; OR, odds ratio; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; 3′-UTR, 3′-untranslated region.

In human, whatever the homologous recombination or non-homologous end-joining pathway, the initial step is to recognize DSBs by the MRE11/RAD50/NBS1 complex (MRN) due to its DNA-binding capability (19). Although the exact mechanisms of how MRN sense and recognize DSBs remain unclear, evidences have indicated that Nijmegen breakage syndrome 1 (NBS1, MIM#251260) protein plays decisive roles in DSBs early responses. On one hand, NBS1 is essential for the assembly of MRN complex at DNA damage site and the activity of MRN function (20,21). NBS1 is also needed for recruiting the relevant phosphoinositide-3 kinase-related kinases, ataxia telangiectasia-mutated (ATM), ATM and Rad3-related (ATR) and DNA-dependent protein kinase catalytic subunit to active the ATM-dependant DNA damage signaling pathway (22). On the other hand, NBS1 participates in cell cycle checkpoints through the ATM/NBS1/SMC1 pathway and the ATM/FANCD2 pathway (23–25). Moreover, NBS1 has been shown to regulate p53-independent apoptosis via an interaction with Ku proteins (26). In addition, mutations in the *NBS1* gene have been identified to cause human disorders such as Nijmegen breakage syndrome, a rare autosomal recessive disease that could cause radiosensitivity and DNA repair deficient, and further increased risk for cancer (27). The germ line homozygous mutation (657del5) of the *NBS1* gene is also believed to promote tumorigenesis (10,28). All these support NBS1 function as a tumor suppressor gene.

Human *NBS1* gene is located at chromosome 8q21, with 754 aa, 4666 bp messenger RNA (mRNA) including 2246 bp 3′-untranslated region (3′-UTR) (Figure 1A). The *NBS1* gene is high polymorphic with 675 single-nucleotide polymorphisms (SNPs), and several polymorphisms in it have been identified to be associated with various cancer risks including lung cancer. However, most of the studied SNPs were limited to those exon variants such as E185Q (15,29). Recently, SNPs in the 3′-UTR of *NBS1* have been reported having associations with cancer risks; two SNPs (rs1063054 and rs1063053) were identified to be associated with risk of lung cancer in a Los Angeles study and risk of non-Hodgkin lymphoma in a south Asian study, respectively (30,31). Because the 3′-UTR plays a great role in gene's mRNA stability, translation efficiency and eventual protein expression, polymorphisms at the microRNA-binding sites may influence the binding ability of microRNA and its posttranscription modulation on gene expression and thus contribute to disease susceptibility; we hypothesized that the polymorphisms in the 3′-UTR of *NBS1* are associated with lung cancer risk by affecting gene's expression.

In two independent hospital-based case–control studies conducted in Southern and Eastern Chinese populations between March 2007 and April 2010, we genotyped three tagSNPs (rs2735383, rs13312986 and rs14448) in the 3′-UTR of *NBS1* gene and analyzed the association between the genetic variations and lung cancer risk. Consecutive functional assays were further performed to determine the biological effects of these polymorphisms.

Materials and methods

Study subjects

We conducted two independent case–control studies in Southern Chinese and Eastern Chinese populations as a discovery set and a validation set, respectively, as described previously (32–34). In brief, 1056 histopathologically confirmed cases with primary lung cancer were recruited at four urban hospitals and at one suburb hospital of Guangzhou city from March 2007 to March 2009, with an overall response rate of about 95%. Thousand and fifty-six age (±1 years) and sex frequency-matched cancer-free controls were randomly picked from ~10 000 participants of healthy check-up programs conducted in Guangzhou City during the same period with a response rate of 84%. An Eastern Chinese population was used to validate the findings with 503 newly

Breast Cancer Res Treat (2011) 126:487–495
DOI 10.1007/s10549-010-1094-6

EPIDEMIOLOGY

A non-synonymous polymorphism *Thr115Met* in the *EpCAM* gene is associated with an increased risk of breast cancer in Chinese population

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Received: 21 July 2010 / Accepted: 23 July 2010 / Published online: 4 August 2010

Abstract As a tumor-associated antigen and a surface marker of breast cancer stem cells (BCSCs), epithelial cell adhesion molecule (EpCAM) plays an important role in not only cell adhesion, morphogenesis, metastases but also carcinogenesis. A non-synonymous C/T polymorphism (rs1126497) in exon3 of *EpCAM* causes a transition of 115 amino acid from Met to Thr. Another polymorphism (A/G, rs1421) in the 3′UTR causes loss of has-miR-1183 binding. A multiple independent case–control analysis was performed to assess the association between *EpCAM* genotypes and breast cancer risk. We observed that the variant *EpCAM* genotype (rs1126497 CT, and TT) was associated with substantially increased risk of breast cancer. Genotyping a total of 1643 individuals with breast cancer and 1818 control subjects in Eastern and Southern Chinese populations showed that rs1126497 CT + TT genotype had an odd ratio of 1.40 (95% confidence interval, 1.16–1.57) for developing breast cancer compared with CC genotype. The allele T increases the risk of breast cancer in a dose-dependent response manner ($P_{trend} < 0.001$). Moreover, compared to breast cancer patients carrying the CC genotype, the *EpCAM* SNP rs1126497 CT or TT carrier was significantly associated with early breast cancer onset ($P = 0.0023$). However, no significant difference was found in genotype frequencies at the rs1421 A/G site between cases and controls. These findings suggest that M115T polymorphism in *EpCAM* may be a genetic modifier for developing breast cancer.

Keywords Breast cancer · Molecular epidemiology · EpCAM · Polymorphisms

Abbreviations

BMI	Body mass index
CI	Confidence interval
EpCAM	Epithelial cell adhesion molecule
MAF	Minor allele frequency
OR	Odds ratio
SNP	Single nucleotide polymorphism

Introduction

Breast cancer is the second most common type of non-skin cancer (after lung cancer) and the fifth most common cause of cancer death. It occurs about 100 times in women than in men but with equal survival rates. Some etiologic factors for breast cancer well-established by epidemiological studies include some environmental factors, such as ionizing radiation [1], high-fat diets [2], alcohol consumption, [3] and use of hormone replacement or oral contraceptives [4]. However, it is suggested that genetic susceptibility plays a more important role in an individual's risk of

L. Jiang, C. Zhang, Y. Li, and X. Yu contributed equally to this work.

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A Practical Synthesis of 2-Aroylindoles from *N*-(2-Formylphenyl)trifluoroacetamides in PEG-400

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Received 18 November 2010; revised 11 January 2011

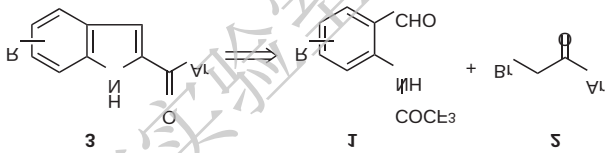
Abstract: A one-pot and environmentally benign approach to the synthesis of highly functionalized 3-unsubstituted 2-aroylindoles is described. Moderate to good yields were obtained through the reaction of easily accessible *N*-(2-formylphenyl)trifluoroacetamides and α-bromoacetophenones in the presence of K_2CO_3. PEG-400 was found to be an efficient and reusable solvent in the process.

Key words: 2-aroylindoles, PEG-400, *N*-(2-formylphenyl)trifluoroacetamides, heterocycles, cyclization

The indole skeleton has been referred to as a 'privileged structure' owing to its diverse biological activities.[1] Particularly, free-NH 3-unsubstituted 2-aroylindoles were reported to be highly potent tubulin polymerization inhibitors,[2] histone deacetylase class I/II inhibitors,[3] platelet-derived growth factor (PDGF) receptor kinase inhibitors,[4] peroxisome proliferator-activated receptor (PPAR) agonists,[5] and cyclooxygenase-2 (COX-2) inhibitors.[6] Different methods have been developed for their syntheses. Among these transformations, addition of acyl electrophiles to 2-lithioindole species[7] and palladium-catalyzed coupling reactions[8] are widely used processes. In other cases, isatins also lead to 2-aroylindoles.[9] Jones reported the preparation of 2-benzoylindole by reaction of 2-aminobenzaldehyde ethylene acetal with α-bromoacetophenone.[10a] However, these synthetic approaches sometimes suffer from harsh reaction conditions, the need for transition-metal assistance, poor availability of starting materials, or long reaction steps. In light of these challenges, a direct and practical method for the formation of 3-unsubstituted 2-aroylindoles is still in demand.

o-Aminoacetophenones reacted readily with α-bromoacetophenones to afford 3-methyl-2-aroylindoles.[10] However, the reaction was limited to *o*-aminoacetophenones in substrate scope, and *o*-aminobenzaldehyde failed to generate 3-unsubstituted aroylindoles.[10a] Our preliminary experiments also showed the failure of this reaction.[11] In a recent patent report, pyrrolo[2,3-*d*]pyrimidines were prepared from 4-(phenylamino)pyrimidine-5-carbaldehydes and α-bromoactophenones.[12] To the best of our knowledge, there is no other report on the analogous indole formation from *o*-aminobenzaldehyde and α-bromoacetophenone.

Herein, we report a convenient and environmentally benign method for the construction of 3-unsubstituted 2-aroylindoles **3** from *N*-(2-formylphenyl)trifluoroacetamides **1**, which can be readily prepared from *o*-aminobenzaldehydes with trifluoroacetic anhydride, and α-bromoacetophenones **2** in PEG-400 (Scheme 1).



Scheme 1 Approach to synthesis of 2-aroylindoles.

N-(2-Formylphenyl)trifluoroacetamide (**1a**) and α-bromoacetophenone (**2a**) were used as model compounds to assess the feasibility of the method. A variety of reaction conditions were examined and the results are shown in Table 1.

When a mixture of **1a** and **2a** was heated in the presence of K_2CO_3 in DMF and PEG-400, 2-benzoylindole (**3a**) was obtained in moderate yields. Moreover, use of PEG-400 as the solvent gave the highest yield (Table 1, entries 1–5). Cs_2CO_3 and K_3PO_4 performed poorly under the reaction conditions (entries 6 and 7). Upon adjusting the ratio of **1a** to **2a** to 1:1.2, an improved yield was observed (entries 5, 8, and 9). Further investigation of reaction time and temperature showed that three hours and 100 °C offered the best result for this transformation (entries 10–13). Thus, the optimized reaction conditions for the formation of **3a** were established (Table 1, entry 8).

PEG-400 is inexpensive and is known to be thermally stable, non-toxic, and is often used as a recyclable reaction medium.[13] Thus, the reusability of PEG-400 in the reaction was examined. After the initial reaction with **1a** and **2a**, the reaction mixture was extracted with diethyl ether and the PEG-400 layer was subjected to a subsequent reaction run by adding further substrates (**1a**, **2a**, and K_2CO_3).[14] The results of the initial and the subsequent three runs of the reaction were consistent in yields (**3a**; 77, 76, 75, and 75%).

Various *N*-(2-formylphenyl)trifluoroacetamides **1** and α-bromoacetophenones **2** were then applied to investigate

SYNTHESIS 2011, No. 6, pp 0873–0880
Advanced online publication: 18.02.2011
DOI: 10.1055/s-0030-1258445; Art ID: F20910SS

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2011, p. 3039–3042 Vol. 55, No. 6
0066-4804/11/$12.00 doi:10.1128/AAC.00085-11

Antimalarial Effects of Human Immunodeficiency Virus Protease Inhibitors in Rhesus Macaques[▽]

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Received 21 January 2011/Returned for modification 22 February 2011/Accepted 29 March 2011

The antimalarial activity of the human immunodeficiency virus protease inhibitors indinavir and saquinavir was evaluated in rhesus macaques for the first time. Indinavir effectively suppressed the growth of *Plasmodium cynomolgi* and *Plasmodium knowlesi in vivo* after a 7- or 3-day treatment, respectively, with clinically relevant doses, whereas saquinavir showed only weak activity against *P. cynomolgi*.

Malaria is one of the most prevalent infectious diseases worldwide, affecting approximately 225 million people, of whom 781,000 died in 2009 (24). Malaria is endemic mainly in sub-Saharan Africa, Southeast Asia, and South America, where human immunodeficiency virus (HIV) infection and AIDS are also prevalent. Some HIV protease inhibitors (HIV PIs), a class of highly active antiretroviral therapy (HAART) drugs, inhibited the proliferation of some laboratory clones and clinical isolates of *Plasmodium falciparum* and *Plasmodium vivax in vitro* and *ex vivo* (1, 15–17, 20, 21) and also showed inhibitory activity against *Plasmodium chabaudi* and *Plasmodium yoelii* in a murine model (1, 13). However, the antimalarial effect of HIV PIs in humans is still unknown.

Plasmodium cynomolgi and *Plasmodium knowlesi* are two species of monkey malaria parasites and are closely related to *P. vivax* (5, 23). The pathology and clinical symptoms of a *P. cynomolgi* infection in monkeys are similar to those of a *P. vivax* infection in humans (23); *P. knowlesi* causes a lethal infection in macaques and severe quotidian malaria in humans (3, 9, 14). Based on the previous studies and our unpublished data, indinavir (15a), ritonavir, and saquinavir behaved synergistically with chloroquine and mefloquine against malaria *in vitro* and in a rodent model (10–12, 20). In the current study, we used *P. cynomolgi* or *P. knowlesi* infection in rhesus macaques to evaluate the antimalarial activities of two of the HIV PIs: indinavir (Crixivan) and saquinavir (Invirase). The monkeys were free of malaria parasites, simian immunodeficiency virus, B virus, D-type simian retrovirus, simian T-lymphotropic virus type-1, and the tubercle bacillus and were housed at the Non-human Primate Animal Center of the Guangzhou Institutes of Biomedicine and Health (GIBH). Experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals. Animal protocols were approved by the GIBH Institutional Animal Care and Use Committee.

First, we evaluated the antimalarial activity of indinavir at different dosages in *P. cynomolgi*-infected monkeys. Twelve 3- to 4-year-old Chinese-origin rhesus macaques (*Macaca mulatta*) were each inoculated intravenously with 1×10^7 *P. cynomolgi*-parasitized erythrocytes thawed after liquid nitrogen storage. Body temperature measurements were taken daily, and parasitemia was recorded daily by examining Giemsa-stained thick and thin blood smears. All monkeys had fever when the parasitemia reached 0.73% to 5.6% (parasite density, presented as the percentage of infected erythrocytes among the total red blood cell [RBC] count) 5 to 7 days after inoculation. Beginning on day 8, three doses of indinavir (20, 40, and 80 mg/kg of body weight dissolved in saline buffer) and the control saline buffer were administered (three monkeys per group) intragastrically three times daily (TID) for seven consecutive days. All the monkeys administered 40 mg/kg or 80 mg/kg indinavir experienced a rapid normalization of body temperature (from high fever to normal temperature, data not shown) and a significant decrease in parasitemia (to less than 0.5%) (Fig. 1A and B) during the initial 3-day treatment. The parasite densities in five of the six monkeys decreased to undetectable levels after a 7-day treatment, and these levels were maintained for 3 to 7 days after termination of the therapy, at which point there was a recrudescence of infection. The monkeys treated with the 20-mg/kg dose underwent some decrease in parasitemia after a 5-day treatment (Fig. 1C) but without resolution of fever during the treatment. The control monkeys treated with the saline buffer experienced high levels of parasitemia (Fig. 1D) and the typical pattern of periodic high fever (data not shown). One monkey in the 20-mg/kg-dose group and two monkeys in the control group required treatment with artesunate to prevent death due to high parasitemia (Fig. 1C and D). At the endpoint of the experiment (day 25), chloroquine was administered (25 mg/kg of body weight per day for 3 days) to terminate the infection (data not shown).

Next, we compared the antimalarial activities of indinavir and saquinavir. Nine monkeys were inoculated with *P. cynomolgi* as described above and divided into three groups (three

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▽ Published ahead of print on 12 April 2011.

OPEN ACCESS Freely available online

PLoS one

Antitumor Effect of Malaria Parasite Infection in a Murine Lewis Lung Cancer Model through Induction of Innate and Adaptive Immunity

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Abstract

Background: Lung cancer is the most common malignancy in humans and its high fatality means that no effective treatment is available. Developing new therapeutic strategies for lung cancer is urgently needed. Malaria has been reported to stimulate host immune responses, which are believed to be efficacious for combating some clinical cancers. This study is aimed to provide evidence that malaria parasite infection is therapeutic for lung cancer.

Methodology/Principal Findings: Antitumor effect of malaria infection was examined in both subcutaneously and intravenously implanted murine Lewis lung cancer (LLC) model. The results showed that malaria infection inhibited LLC growth and metastasis and prolonged the survival of tumor-bearing mice. Histological analysis of tumors from mice infected with malaria revealed that angiogenesis was inhibited, which correlated with increased terminal deoxynucleotidyl transferase-mediated (TUNEL) staining and decreased Ki-67 expression in tumors. Through natural killer (NK) cell cytotoxicity activity, cytokine assays, enzyme-linked immunospot assay, lymphocyte proliferation, and flow cytometry, we demonstrated that malaria infection provided anti-tumor effects by inducing both a potent anti-tumor innate immune response, including the secretion of IFN-γ and TNF-α and the activation of NK cells as well as adaptive anti-tumor immunity with increasing tumor-specific T-cell proliferation and cytolytic activity of $CD8^+$ T cells. Notably, tumor-bearing mice infected with the parasite developed long-lasting and effective tumor-specific immunity. Consequently, we found that malaria parasite infection could enhance the immune response of lung cancer DNA vaccine pcDNA3.1-hMUC1 and the combination produced a synergistic antitumor effect.

Conclusions/Significance: Malaria infection significantly suppresses LLC growth via induction of innate and adaptive antitumor responses in a mouse model. These data suggest that the malaria parasite may provide a novel strategy or therapeutic vaccine vector for anti-lung cancer immune-based therapy.

Citation: Chen L, He Z, Qin L, Li Q, Shi X, et al. (2011) Antitumor Effect of Malaria Parasite Infection in a Murine Lewis Lung Cancer Model through Induction of Innate and Adaptive Immunity. PLoS ONE 6(9): e24407. doi:10.1371/journal.pone.0024407

Editor: Hong Wei Chu, National Jewish Health, United States of America

Received May 18, 2011; **Accepted** August 8, 2011; **Published** September 9, 2011

Funding: Grant support from the National Natural Sciences Foundation of China (No. 81001022 to ZH), a grant from the State Key Laboratory of Respiratory Disease to XC, and a grant from the CAS-FS Biotech and Pharmaceutical Center to XC. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. Although treatment methods in surgery, irradiation, and chemotherapy have been improved, prognosis remains unsatisfactory, and developing new therapeutic strategies is still an urgent demand. Immunotherapy may represent one of new therapeutic strategies for lung cancer has recently been developed [2–5]. The goal of lung cancer immunotherapy is to augment the weakened host immune response against tumors using specific and/or nonspecific immune stimulants [4–6]. Nonspecific immunostimulatory agents and interventions with cytokines have limited clinical benefits. The target-directed immunotherapy with defined tumor antigens, such as melanoma-associated antigen 3 and mucin 1(MUC1), are suboptimal and strong adjuvant agents are needed [6,7]. In addition, it is now clear that lung cancer often present a tolerogenic microenvironment that hampers effective antitumor immunity. Therefore, new potent and efficacious immunotherapy, both augmenting antitumor immunity and counteracting tumor-mediated immunosuppression for lung cancer are needed.

Malaria, which is caused by an intracellular parasite from the *Plasmodium* genus, is the most common parasitic infection in humans. Human malaria parasite infection can produce periodic high fevers in the acute phase. Hyperthermia has been clinically used for the treatment of certain cancers [8–11]. Furthermore, malaria has been reported to stimulate host immune responses, such as promoting IFN-γ production, activating natural killer (NK)

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PLoS one

Automated Analysis of Time-Lapse Imaging of Nuclear Translocation by Retrospective Strategy and Its Application to STAT1 in HeLa Cells

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Abstract

Cell-based image analysis of time-lapse imaging is mainly challenged by faint fluorescence and dim boundaries of cellular structures of interest. To resolve these bottlenecks, a novel method was developed based on "retrospective" analysis for cells undergoing minor morphological changes during time-lapse imaging. We fixed and stained the cells with a nuclear dye at the end of the experiment, and processed the time-lapse images using the binary masks obtained by segmenting the nuclear-stained image. This automated method also identifies cells that move during the time-lapse imaging, which is a factor that could influence the kinetics measured for target proteins that are present mostly in the cytoplasm. We then validated the method by measuring interferon gamma (IFNγ) induced signal transducers and activators of transcription 1 (STAT1) nuclear translocation in living HeLa cells. For the first time, automated large-scale analysis of nuclear translocation in living cells was achieved by our novel method. The responses of the cells to IFNγ exhibited a significant drift across the population, but common features of the responses led us to propose a three-stage model of STAT1 import. The simplicity and automation of this method should enable its application in a broad spectrum of time-lapse studies of nuclear-cytoplasmic translocation.

Citation: Han F, Liang P, Wang F, Zeng L, Zhang B (2011) Automated Analysis of Time-Lapse Imaging of Nuclear Translocation by Retrospective Strategy and Its Application to STAT1 in HeLa Cells. PLoS ONE 6(11): e27454. doi:10.1371/journal.pone.0027454

Editor: Barbara Mellone, University of Connecticut, Storrs, United States of America

Received July 4, 2011; **Accepted** October 17, 2011; **Published** November 18, 2011

Funding: This study was supported by National Natural Science Foundation of China (No. 30870535 and 90913017). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Introduced Innovative R&D Team Program of Guangdong Province (Gene Silencing Technology and Therapeutics).

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The movement of proteins such as transcription factors between the cytoplasm and nucleus is of great biological importance in many signaling pathways [1]. Time-lapse imaging of proteins that shuttle between nuclei and cytoplasm is also an area of increasing interest to systems biologists who are tracking protein behaviors in cells over time for modeling [2]. The most advantageous is to study the native state of cells with minimal distortions of cell morphology or function. However, most automated image analysis systems currently perform well only with fixed cells [3,4,5,6]. Such experiments can also be analyzed manually, but the volume and complexity of the data generated are huge.

Some methods [7,8], including software, such as CellTracker [6], introduce different strategies applicable for time-lapse imaging of nuclear-cytoplasmic translocation of fluorescently tagged proteins. Most of these studies focus on improving the possibilities for image analysis and hence present two major limitations. Firstly, the algorithms are often too profound for users to interpret, leading to difficulties in the applications. Secondly, image processing may be difficult under circumstances, such as incomplete nuclear-cytoplasmic translocation which causes ambiguous nuclear boundaries, or faint cellular fluorescence. Also, cells, particularly transiently transfected cells, may display fluorescence that varies significantly in intensity. These phenomena are common in live-cell imaging, but all create difficulties for differentiating nuclei from cytoplasm, even manually. Indeed, very few automated image analysis techniques can potentially satisfy the requirements imposed by live cell imaging and analysis at the individual cell level [9,10,11]. To the best of our knowledge, there is no system available which enables to track and identify a large volume of dynamic cellular image data of protein nuclear transport automatically and effectively.

The first crucial step to differentiate nuclei from cytoplasm is achieved by image segmentation [12,13,14]. Convincing segmentation requires images with high contrast, which is sometimes difficult to achieve in live-cell imaging, but much easier in fixed-cell imaging. For cells that undergo little morphological change during a time-lapse experiment, it is feasible to perform "retrospective" analysis (Fig. 1A). In this analysis, cells are fixed and stained at the end of an experiment to acquire high-contrast images, which are segmented into binary masks to process the time-lapse images (Fig. 1B). Two questions then arise and the method presented here resolves both. One is how to find the same field after fixing the cells. The equipment of XY positioning stages in imaging platforms such as slide-based cytometry [15], together

DOI: 10.1002/adsc.201100223

Brønsted Acid-Promoted Sequential Hydroarylation–Hydroamidation of Arene-Tethered 1-(2-Alkynylphenyl)ureas: Direct Access to 4,4-Spiro-3,4-dihydro-2-(1*H*)-quinazolinones

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Received: March 28, 2011; Revised: May 9, 2011; Published online: September 29, 2011

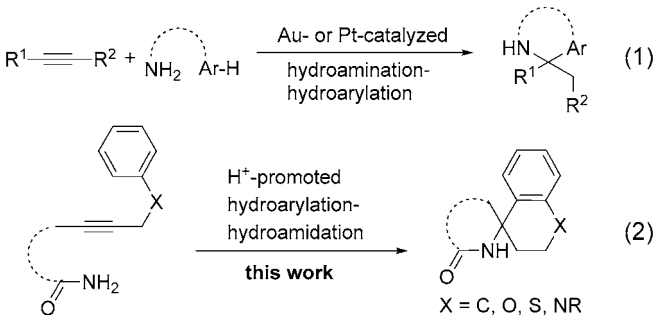
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adcs.201100223.

Abstract: A Brønsted acid-promoted approach to 4,4-sipro-3,4-dihydro-2-(1*H*)-quinazolinones from arene-tethered 1-(2-alkynylphenyl)ureas has been developed. The reaction is initiated by intramolecular hydroarylation followed by hydroamidation, assisted by intramolecular proton transfer from the protonated urea moiety. A variety of tethering atoms, including carbon, oxygen, sulfur, and protected nitrogen are compatible with the reaction conditions.

Keywords: acidity; alkynes; cyclization; heterocycles; hydroamidation; hydroarylation

Considerable attention has been paid to cascade reactions involving alkynes, in which multiple carbon-carbon and/or carbon-heteroatom bonds are formed sequentially in one operation, enabling a fast assembly of complex and diversified molecular architectures.[1] Transition metal- or Brønsted acid-promoted direct addition of Ar–H bonds or N–H bonds across alkynes or alkenes, known as hydroarylation[2] and hydroamination[3], provides atom-economical and step-efficient processes to functionalized arenes and N-containing compounds. Compared with double hydroarylation[4] and double hydroamination[5], cascade hydroamination–hydroarylation or hydroarylation–hydroamination on the same carbon-carbon triple bond is relatively undervalued. Recently, Patil and co-workers reported Pt-catalyzed hydroamination–hydroarylation cascade reactions to construct complex multi-ring N-heterocyclic compounds.[6] In a report by Dixon et al, Au(I)-catalyzed a formal hydroamination–hydroarylation reaction of alkynoic acids and pyrrole or indole-substituted ethylamines through *N*-acyliminium intermediates [Eq. (1), Scheme 1].[7] Brønsted acid-promoted hydroamination is less common because, in most cases, nitrogen is more basic than the π-systems of alkynes or alkenes, which makes the nitrogen non-nucleophilic upon protonation.[3] The use of Brønsted acid in cascade hydroamination–hydroarylation is even more scarce in theliterature.[8] Herein, we would like to disclose a Brønsted acid-promoted synthesis of a unique spirocyclic N-heterocycle by locating the amide and the arene moieties at separate ends of an alkyne [Eq. (2), Scheme 1].

With this idea in mind, we designed compound **1a**,[9] a phenol-tethered 1-(2-alkynylphenyl)urea using the phenolic oxygen as the linker, to test our hypothesis (Table 1). It was anticipated that both the low basicity of the urea nitrogen and the high electron density of the tethered phenol will facilitate the process of Brønsted acid-promoted hydroamidation and hydroarylation. To our delight, a unique spiroquinazolinone derivative **2a** was isolated in excellent yield (87%) under the conditions involving 1.5 equivalents of TfOH in refluxing DCE (entry 1, Table 1). A series of spiroquinazolinone derivatives were claimed as promising PDE7 inhibitors.[10] Methods for the synthesis of



Scheme 1. Cascade hydroamination–hydroarylation and hydroarylation–hydroamidation of alkynes.

Virus Research 155 (2011) 156–162

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Comparative immunogenicity of recombinant adenovirus-vectored vaccines expressing different forms of hemagglutinin (HA) proteins from the H5 serotype of influenza A viruses in mice

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ARTICLE INFO

Article history:
Received 16 June 2010
Received in revised form
20 September 2010
Accepted 21 September 2010
Available online 29 September 2010

Keywords:
HPAI
Immunogenicity
Recombinant adenovirus vector
Influenza vaccine

ABSTRACT

Recent outbreaks of highly pathogenic avian influenza (HPAI) H5N1 viruses in poultry and their subsequent transmission to humans have highlighted an urgent need to develop preventive vaccines in the event of a pandemic. In this paper we constructed recombinant adenovirus (rAd)-vectored influenza vaccines expressing different forms of H5 hemagglutinin (HA) from the A/Vietnam/1194/04 (VN/1194/04) virus, a wild-type HA, a sequence codon-optimized HA and a transmembrane (TM) domain-truncated HA. Compared to the rAd vectors expressing the wild-type HA (rAd-04wtHA) and the TM-truncated form of HA (rAd-04optHA-dTM), the rAd vectored vaccine with the sequence codon-optimized HA (rAd-04optHA) showed a tendency to induce much higher hemagglutinin inhibition (HI) antibody titers in mice immunized with a prime-boost vaccine. Furthermore, administration of the rAd-04optHA vaccine to mice could elicit cross-reactive immune responses against the antigenically distinct HK/482/97 virus. Additionally, we constructed another vector containing the codon-optimized HA of the A/Hong Kong/482/97 (HK/482/97) virus. Administration of a bivalent immunization formulation including the rAd-04optHA and rAd-97optHA vaccines to mice induced a stronger immune response against HK/482/97 virus than the monovalent formulation. Taken together, these findings may have some implications for the development of rAd-vectored vaccines in the event of the pandemic spread of HPAI.

1. Introduction

When it first emerged in 1997, the highly pathogenic avian influenza virus (HPAI) H5N1 strain caused widespread death in domestic and wild birds, and it continued to cross species barriers to infect humans and other mammals, resulting in 18 infected people and six deaths (de Jong et al., 1997; Subbarao et al., 1998). Since 2003, highly pathogenic H5N1 influenza viruses have spread across the globe and caused highly fatal disease (Peiris et al., 2004; World Health Organization, 2010). Most human infections are usually ascribed to direct transmission of the virus from infected poultry to humans, and very few cases have yet been transmitted from person to person. However, extensive exposure of humans to H5N1 viruses increases the possibility of adaptation of the virus that may be transmitted efficiently within the global human population through genetic mutation or avian–human reassortment (Li et al., 2004; Ungchusak et al., 2005). Concerns about the potential for the generation of a pandemic H5 strain and its concomitant morbidity and mortality underscore the importance of H5N1 influenza virus research and pandemic preparedness.

Currently, the best option for combating the impact of influenza virus infection in humans is vaccination (Gambotto et al., 2008; Cinatl et al., 2007; Subbarao and Joseph, 2007). The high pathogenicity of the H5N1 viruses in human beings and poultry presents specific difficulties for the conventional inactivated vaccine preparation. The viruses must be handled under biosafety level (BSL)-3+ conditions, and they cannot grow efficiently in embryonated eggs, the standard medium for reassortment and propagation before the inactivation of influenza viruses used for vaccines (Hoffmann et al., 2005; Lipatov et al., 2005; Tian et al., 2005; Webby et al., 2004). To overcome the high pathogenicity of the viruses, a new plasmid-based reverse genetics technology has been developed to generate high-growth reassortants that combine the high-growth-yield strain of influenza A virus, A/PR/8/34, with expression of the HA and NA antigenic glycoproteins from the target viral strains (Hoffmann et al., 2002, 2000; Neumann et al., 2005, 1999). The HA gene is modified to eliminate virulence, which is primarily determined by the multibasic cleavage site sequence, through reverse genetics, permitting the virus to grow well in eggs (Horimoto et al., 2006; Li et al., 1999; Liu et al., 2003;

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0168-1702/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2010.09.014

VIROLOGY JOURNAL

SHORT REPORT Open Access

Complete genome analysis of a novel E3-partial-deleted human adenovirus type 7 strain isolated in Southern China

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Abstract

Human adenovirus (HAdV) is a causative agent of acute respiratory disease, which is prevalent throughout the world. Recently there are some reports which found that the HAdV-3 and HAdV-5 genomes were very stable across 50 years of time and space. But more and more recombinant genomes have been identified in emergent HAdV pathogens and it is a pathway for the molecular evolution of types. In our paper, we found a HAdV-7 GZ07 strain isolated from a child with acute respiratory disease, whose genome was E3-partial deleted. The whole genome was 32442 bp with 2864 bp deleted in E3 region and was annotated in detail (GenBank: HQ659699). The growth character was the same as that of another HAdV-7 wild strain which had no gene deletion. By comparison with E3 regions of the other HAdV-B, we found that only left-end two proteins were remained: 12.1 kDa glycoprotein and 16.1 kDa protein. E3 MHC class I antigen-binding glycoprotein, hypothetical 20.6 kDa protein, 20.6 kDa protein, 7.7 kDa protein., 10.3 kDa protein, 14.9 kDa protein and E3 14.7 kDa protein were all missing. It is the first report about E3 deletion in human adenovirus, which suggests that E3 region is also a possible recombination region in adenovirus molecular evolution.

Introduction

Human adenoviruses (HAdVs) are implicated in a wide range of human diseases, including respiratory, ocular, metabolic, renal and gastrointestinal. They are responsible for 5-10% of lower respiratory tract infections in infants and children throughout the world. HAdV-7, a member of the B1 subspecies, causes acute respiratory disease (ARD). This pathogen is identified in epidemics, is highly virulent and is associated with clinical manifestations of considerable severity including residual lung damage and fatal outcomes [1]. Previous reports suggested that HAdV-3 and -5 are very stable across 50 years of time and space [2,3], which is common in DNA viruses. But HAdV in general are known to undergo recombination. Earlier studies demonstrated in vitro recombination. But more and more isolates, which were isolated from adenovirus epidemic, undergo new recombination between adenovirus types, which leaded to new"intermediates" or subtypes [4]. All the evidence supports the hypothesis that genome recombination drives the molecular evolution of HAdV types. In our research, we found a HAdV-7 strain isolated from a child with acute respiratory disease, with a large portion of E3 region deleted. The whole genome was annotated (GenBank: HQ659699). It hints that E3 region is also important in adenovirus recombination and molecular evolution.

Materials and methods

1. Cells, virus and Preparation of viral DNA

The virus strain (designated HAdV-7 GZ07) in this study was isolated from nasal aspirates of a child with ARD in southern China in 2007. The Nasal aspirate specimen was inoculated to HEp-2 cells for isolation, which was maintained in minimal essential medium supplemented

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Virus Research 160 (2011) 214–220

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Construction and characterization of human adenovirus serotype 3 packaged by serotype 7 hexon

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ARTICLE INFO

Article history:
Received 10 February 2011
Received in revised form 16 June 2011
Accepted 22 June 2011
Available online 29 June 2011

Keywords:
Chimera
Hexon
Fiber
Human adenovirus
Serotype
Hypervariable regions

ABSTRACT

Human adenovirus serotype 3 (Ad3) and serotype 7 (Ad7) are important pathogens causing respiratory tract diseases such as acute respiratory disease in pediatric and adult patients, but the immunodominant targets of Ad3- and Ad7-specific neutralizing antibodies (NAbs) remain unclear. A chimeric Ad vector, Ad3/H7, was constructed by replacing the Ad3 hexon gene (H3) with the hexon gene (H7) of Ad7. The chimeric viruses were successfully rescued in HEp-2 cells, and the Ad7 hexon was able to encapsidate the Ad3 genome, and functioned as efficiently as the Ad3 hexon. Furthermore, we tested the host neutralization responses against the viruses using BALB/C mice. Up to 97% of the NAbs produced by mice that were infected with these viruses were specific for the hexon protein in vitro. Preimmunization of mice with one of Ad7 and Ad3/H7 significantly prevented subsequent intranasal infection of the other type in vivo. In contrast, preimmunization of mice with one of Ad3 and Ad3/H7 did not remarkably prevent subsequent infection of the other type. We next evaluated the functional significance of hexon and other structural proteins specific NAbs to suppress the immunogenicity of Ad3/H3 and Ad3/H7 vectors expressing EGFP in mice preimmunized with wild type Ad. Preimmunization of mice with Ad7 evidently suppressed EGFP-specific humoral immune responses elicited by Ad3/H7, and did not exert suppressive effects on Ad3/H3. But contrary to the in vitro neutralization results, EGFP-specific humoral immune responses elicited by Ad3/H7 was remarkably inhibited in Ad3-preimmunization mice. The whole genome of the Ad7 strain was sequenced and aligned with Ad3. The major differences between Ad3 and Ad7 were only observed in the fiber and hexon among all structural proteins, and the variation between the hexons only located in four hypervariable regions (HVRs), HVR-1, -2, -5, and -7. These results thus suggest that Ad3- and Ad7-specific NAbs are directed primarily against the hexon proteins both in vitro and in vivo. But high titer Ad3 fiber-specific NAbs may also play an important role in blunting Ad3 immunogenicity in vivo. These studies contribute to a more profound understanding of Ad immunogenicity and have relevance for the design of novel Ad vaccine.

1. Introduction

Human adenoviruses (HAdVs) in the alimentary canal and respiratory tract were discovered in 1953, and have been found to cause a broad spectrum of diseases in pediatric and adult patients (Aoki and Tagawa, 2002; Arnold et al., 2010; Kunz and Ottolini, 2010; Louie et al., 2008; Rowe et al., 1953). HAdV can be classified into six species (A–F) consisting of 53 serotypes, based on serum neutralization antibodies and nucleotide sequence. Recently, a new serotype, HAdV52, was reported and defined as a seventh species, species G (Davison et al., 2003; Green et al., 1979; Ishiko et al., 2008; Jones et al., 2007; Walsh et al., 2009). Recently, a broad recombination phenomenon has been discovered between different serotypes of virus strains leading to lethal strains, some of which may be new serotypes (Kajon et al., 2010; Lukashev et al., 2008; Rebelo-de-Andrade et al., 2010). Members of species B, HAdV-3 and -7, have caused severe respiratory disease, such as acute respiratory disease (ARD) pediatric pneumonia epidemics and outbreaks in Asia, Europe and America (Erdman et al., 2002; Frantzidou et al., 2005; Hierholzer, 1992; Li et al., 2005; Tang et al., 2011). As yet, there is no effective medicine or vaccine except for live, oral vaccines used by the US military from 1971 to 1996 (Gooch and Mogabgab, 1972; Lyons et al., 2008). For all these reasons, development of an effective vaccine directing HAdV-3 and -7 is required.

An adequate knowledge of the immunodominant regions of adenovirus NAbs will be helpful for the development of novel adenovirus vaccine. The Ad capsid consists of three major structural proteins: hexon, fiber, and penton base. Research carried out on adenovirus serotype 2 (Ad2) and Ad5 have identified the major

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0168-1702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2011.06.017

Oxygen Activation

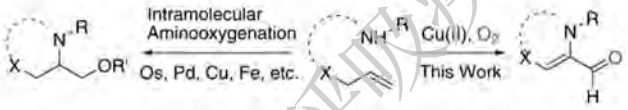
DOI: 10.1002/anie.201100362

Copper-Catalyzed Intramolecular Dehydrogenative Aminooxygenation: Direct Access to Formyl-Substituted Aromatic N-Heterocycles**

Honggen Wang, Yong Wang, Dongdong Liang, Lanying Liu, Jiancun Zhang, and Qiang Zhu**

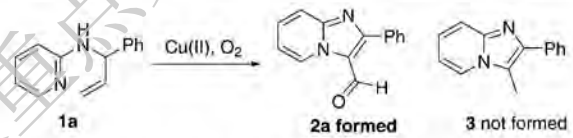
Aminooxygenation of alkenes,[1-3] a process in which nitrogen and oxygen atoms are added simultaneously across a carbon–carbon double bond, represents one of the most straightforward approaches to prepare vicinal amino alcohol derivatives, which are an important functional motif in many biologically active compounds.[4] The regioselective intramolecular version of this process leads to a variety of nitrogen-containing heterocycles,[5] in which an exocyclic oxygenated methylene group is present for further elaboration. In studies focusing on the development of this method, less-toxic metal catalysts, including palladium,[6] copper,[7] and iron,[8] in addition to the toxic osmium salts have been explored.[9] To elaborate the N-heterocycles formed in this fashion, deprotection ($R' \neq H$) and oxidation strategies have been probed. In these efforts, oxidation of the exocyclic primary alcohols to form aldehydes, among the most versatile functional groups in chemical transformations, was found to be a general strategy.[3c,7] Herein, we describe the results of an investigation that has led to the discovery of an unexpected and novel intramolecular dehydrogenative aminooxygenation (IDA) reaction, catalyzed by copper and occurring under dioxygen. The process results in the direct formation of aromatic N-heterocycles substituted with a formyl group (Scheme 1).[10]

The presence of the imidazo[1,2-*a*]pyridine scaffold in many biologically active compounds has stimulated the development of numerous methods for their preparation.[11]



Scheme 1. Intramolecular dehydrogenative aminooxygenation.

Recently, Chernyak and Gevorgyan[12] described a new copper-catalyzed, three-component coupling reaction that was used to generate an impressive array of imidazo[1,2-*a*]pyridine derivatives. By considering features of our recent synthesis of pyrido[1,2-*a*]benzimidazoles through copper-catalyzed aromatic C–H amination of *N*-aryl-2-aminopyridines,[13] we hypothesized that 3-methyl-2-phenylimidazo[1,2-*a*]pyridine **3** would be formed under the developed reaction conditions when *N*-(1-phenylallyl)-2-aminopyrine **1a** is employed as substrate. We envisaged that this transformation would take place either by direct amination of the vinyl C–H bond in **1a** and subsequent double bond migration or through intramolecular hydroamination of **1a** followed by dehydrogenative aromatization (Scheme 2). In contrast to this prediction, the copper-catalyzed reaction of **1a** actually formed 2-phenylimidazo[1,2-*a*]pyridine-3-carbaldehyde **2a**, which is a potentially versatile synthetic intermediate.[14] In this unexpected process, the terminal carbon atom of the monosubstituted olefin moiety in **1a** is transformed into the formyl group with concurrent formation of the N-heterocyclic ring in **2a**. Although imidazo[1,2-*a*]pyridine-3-carbaldehydes can be prepared through Vilsmeier–Haack formylation of the corresponding imidazo[1,2-*a*]pyridines,[14] the extremely low yields (20–30%) and harsh reaction conditions limits the application of this approach.



Scheme 2. Unexpected formation of **2a**.

The widespread distribution of substituted imidazoles in biologically active natural products and synthetic drugs or drug candidates makes them important synthetic targets.[15,16] Owing to the electron-deficient nature of imidazole, its formylation cannot be realized through Vilsmeier–Haack reaction. An alternative deprotonation with BuLi and subsequent nucleophilic addition to DMF at low temperature is accessible.[17] However, deprotonation of 1,2-disubstituted imidazole occurs at the 5-position exclusively, and no direct formylation at the 4-position of 1,2-disubstituted imidazoles has been reported in the literature.[18] Herein, we report the synthesis of imidazo[1,2-*a*]pyridine-3-carbaldehydes as well as 1,2-disubstituted imidazole-4-carbaldehydes through the

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[**] We are grateful for financial support of this work by a Start-up Grant from Guangzhou Institutes of Biomedicine and Health (GIBH), and by the National Science Foundation of China (21072190) and the National Basic Research Program of China (973 Program 2011CB504004 and 2010CB945500). We thank Prof. Jinsong Liu (GIBH) for providing X-ray structural analysis.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201100362.

Experimental Lung Research, 37, 186–194, 2011

ISSN: 0190-2148 print / 1521-0499 online
DOI: 10.3109/01902148.2010.540768

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Cough reflex sensitivity is increased in guinea pigs with parainfluenza virus infection

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ABSTRACT

The purpose of this study was to investigate for the change in cough reflex sensitivity (CRS) caused by parainfluenza virus type 3 (PIV3) infection. Guinea pigs were randomized into a vehicle control, an asthma control, or 1 of 4 PIV3-inoculated groups (referred to as postinfection day [PID] 6, 12, 28, and 42 groups). Evidence of viral protein and nucleic acid within the lung confirmed successful PIV3 infection. Plethysmography was used to assess CRS and airway reaction and airway inflammation was assessed via bronchoalveolar lavage fluid cytology and lung histopathology. Compared with the vehicle control group, CRS was significantly increased in all PID groups ($P < .05$) in concert with an obvious airway hyperresponsiveness in the PID 6 group. Though a small increase in CRS in the asthma control group was noted, it was not significant compared to the vehicle control group. Total cell counts from the bronchoalveolar lavage fluid of all PIV3-inoculated groups increased markedly and the number of lymphocytes was significantly increased in the PID 6 and PID 12 groups. The lung pathology of PIV3-inoculated animals showed airway inflammation without pneumonia in the acute infectious phase. The temporal and spatial variation of CRS may be the essential mechanism of cough caused by PIV3.

KEYWORDS cough, cough reflex sensitivity, guinea pigs, human parainfluenza virus type 3, postinfectious cough

The acute upper respiratory tract infection (URTI) is the most common infectious diseases of humans. Adults typically experience 2 to 4 colds per year and children usually have more [1]. The acute cough, defined as a cough lasting <3 weeks' duration, is most often caused by an URTI [2]. Patients who complain of a persistent cough lasting more than 3 weeks after experiencing acute symptoms of an URTI may have a postinfectious cough [3]. This postinfectious cough is considered a subacute cough if the condition lasts for no more than 8 weeks and is the most common cause of subacute cough [4]. Postinfectious coughs are primarily caused by viral infection and are therefore referred to as postviral coughs. Thoracic radiographic findings are normal in such patients, thereby ruling out pneumonia. Some researchers have confirmed that following an acute URTI, approximately 11% to 25% of patients suffer from a subacute or even chronic cough [3, 5].

Unfortunately, few, if any, effective therapies exist for the acute cough caused by a viral URTI [6] and, to date, no satisfactory treatment options are available for those suffering from a persistent postviral cough. In addition, little information is available regarding the detailed pathogenesis of the postviral cough and animal studies of virus-induced coughing have not been reported. In fact, remarkably few studies that directly investigate the mechanism(s) of viral-induced coughing have been published. Those that have provided some insight about URTI have investigated only airway hyperresponsiveness (AHR) rather than the cough itself. Moreover, investigations that have specifically evaluated cough have not studied experimental viral infections as a cause of cough [7].

Human parainfluenza virus type 3 (PIV3) is one of the major pathogens responsible for URTI in humans, especially pediatric patients. PIV3 alone

Received 13 July 2010; accepted 12 November 2010

The authors wish to thank all members of the State Key Laboratory of Respiratory Disease for their constructive comments and support. This work was supported in part by grant 30700341 from the National Natural Science Foundation.

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FEBS Letters 585 (2011) 1175–1179

FEBS Letters

journal homepage: www.FEBSLetters.org

Crystal structure of E339K mutated human glucokinase reveals changes in the ATP binding site

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ARTICLE INFO

Article history:
Received 31 January 2011
Revised 11 March 2011
Accepted 11 March 2011
Available online 22 March 2011

Edited by Judit Ovádi

Keywords:
Human glucokinase
E339K mutation
Crystal structure
Phosphorylated serine

ABSTRACT

Human glucokinase (GK) plays an important role in glucose homeostasis. An E339K mutation in GK was recently found to be associated with hyperglycemia. It showed lower enzyme activity and impaired protein stability compared to the wild-type enzyme. Here, we present the crystal structure of E339K GK in complex with glucose. This mutation results in a conformational change of His416, spatially interfering with adenosine-triphosphate (ATP) binding. Furthermore, Ser411 at the ATP binding site is phosphorylated and then hydrogen bonded with Thr82, physically blocking the ATP binding. These findings provide structural basis for the reduced activity of this mutant.

1. Introduction

Human glucokinase (GK) (adenosine-triphosphate (ATP): D-glucose 6-phosphotransferase, EC 2.7.1.2) is a 52 kD enzyme that can phosphorylate glucose to glucose-6-phosphate. It is mainly expressed in liver, pancreas, gut and brain [1,2]. Also known as hexokinase IV or D, GK is a member of the hexokinase family that catalyzes the first step in glycolysis, playing a crucial role in glucose homeostasis. However, GK exhibits sigmoidal kinetics instead of the Michaelis–Menten kinetics of non-allosteric hexokinases [3]. It is also different from non-allosteric hexokinases in its lack of product inhibition and relatively lower glucose affinity. In pancreatic β-cells, GK functions as a glucose sensor [4]. In liver, it regulates glucose uptake and storage [5]. Given its key role in glucose homeostasis, mutations of the *GK* gene have profound influence on its functions in human cells. To date, about 600 mutations have been reported for the *GK* gene, including non-sense, missense, and frameshift mutations [6,7]. A large number of GK mutations are inactivating mutations associated with maturity-onset diabetes of the young, type2 (MODY2). MODY2 is a form of diabetes mellitus caused by mutations in an autosomal dominant gene. A number of GK mutations, such as T51I, W99R, Y214C, V455M and A456V, are activating mutations that result in persistent hyperinsulinemic hypoglycemia of infancy (PHHI) [8–11].

In 2004, Katama and colleagues determined the crystal structures of human GK in both active and inactive forms [12]. Their results showed that GK has a small domain and a large domain separated by a deep cleft and undergoes a large conformational change by the rotation of the small domain induced by glucose binding. Their structure provides a general explanation of how the enzyme activity of GK can be affected by some of the mutations, especially those located in the glucose and ATP binding pockets and those involved in the conformation transition. Recently, a new inactivating mutation E339K was found by Shen et al. in a Chinese family with hyperglycemia [13]. This E339K mutation, however, is situated far from the ATP binding site. To understand how this mutation affects GK activity, we determined the crystal structure of E339K mutated GK. This first mutated GK crystal structure will shed light on how the mutation causes the enzyme kinetic alterations and will provide a better understanding on how the enzyme works.

2. Materials and methods

2.1. Protein expression and purification

The E339K GK, with an N-terminal MGHHHHHHENLYFQGM tag and corresponding amino acid residues 12–465, was cloned into

Abbreviations: GK, glucokinase; AMPPNP, adenylyl-imidodiphosphate; SEP, phosphorylated serine; ATP, adenosine-triphosphate; IPTG, isopropyl-β-D-thiogalactoside; DTT, dithiothreitol; TCEP, tris (2-carboxyethyl) phosphine; G6PDH, glucose-6-phosphate dehydrogenase; NADP+, nicotinamide adenine dinucleotide phosphate

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0014-5793/$36.00
doi:10.1016/j.febslet.2011.03.026

Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary crystallographic studies of a cysteine protease inhibitor from the human nematode parasite *Ascaris lumbricoides*

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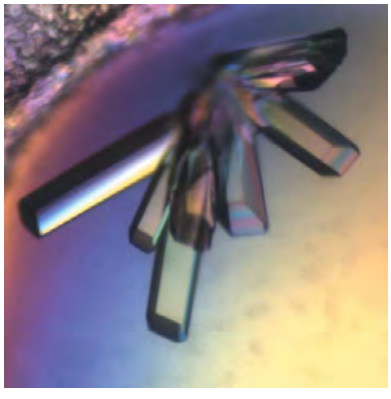
Received 17 October 2010
Accepted 3 December 2010



The cysteine protease inhibitor from *Ascaris lumbricoides*, a roundworm that lives in the human intestine, may be involved in the suppression of human immune responses. Here, the molecular cloning, protein expression and purification, preliminary crystallization and crystallographic characterization of the cysteine protease inhibitor from *A. lumbricoides* are reported. The rod-shaped crystal belonged to space group *C*2, with unit-cell parameters $a = 99.40$, $b = 37.52$, $c = 62.92$ Å, $\beta = 118.26^\circ$. The crystal diffracted to 2.1 Å resolution and contained two molecules in the asymmetric unit.

1. Introduction

The cysteine protease inhibitor (CPI) superfamily comprises a number of proteins that are present across species. CPIs mostly target family C1 (papain-like) cysteine proteases (Turk *et al.*, 1997). The cellular functions of CPIs include protection against unwanted proteolysis and regulation of intracellular and extracellular protein breakdown. For example, cathepsins (a mammalian version of family C1 cysteine proteases) have been shown to be involved in antigen presentation, apoptosis and bone remodeling in addition to their roles in lysosomal protein degradation (Chapman *et al.*, 1997; Honey & Rudensky, 2003; Turk *et al.*, 2002). Cathepsin function must be tightly regulated in order to avoid unnecessary activation. This regulation is provided through the binding of CPIs to cathepsins.

CPIs have been identified in many species of nematode parasites. They are believed to play important roles in the regulation of essential developmental events such as the moulting or the hatching of the worms (Lustigman *et al.*, 1992). Studies in recent years have demonstrated that protease inhibitors from parasitic worms are also able to modulate the functions of host immune systems (Gregory & Maizels, 2008). Further studies have shown that protease inhibitors of parasitic origin may influence the activity of cathepsin S, a cysteine protease that is important in antigen processing and presentation by dendritic cells, resulting in impaired immune responses (Dainichi *et al.*, 2001; Schonemeyer *et al.*, 2001). Owing to their immune-modulating properties, parasite protease inhibitors may have pharmaceutical value for the treatment of autoimmune and allergic diseases in humans.

We observed that a CPI from *Ascaris lumbricoides*, a roundworm that lives in the human intestine, strongly suppresses the activation of human immune cells (unpublished data). In this report, we performed a preliminary X-ray analysis of CPI from *A. lumbricoides* (Al-CPI) with the aim of understanding its three-dimensional structure.

2. Materials and methods

2.1. Molecular cloning, protein expression and purification

Total RNA was isolated from adult *A. lumbricoides* worms. Double-stranded cDNA was obtained by RT-PCR from total RNA using a reverse transcription system (Promega). The gene encoding Al-CPI (GenBank accession No. HQ404231) was amplified by PCR from cDNA with the primers 5′-CCG**GAATTC**GAAAACCTGTA-TTTTCAGGGCCAAGTAGGAGTTCCTGGTGGTTTC-3′ and 5′-ACGC**GTCGAC**TTATGCAGATTTGCATTCTTTGATG-3′ (*Eco*RI and *Sal*I sites, respectively, are shown in bold). The purified PCR

Liu et al. BMC Infectious Diseases 2011, 11:345
http://www.biomedcentral.com/1471-2334/11/345

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern China

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Abstract

Background: Human bocavirus (HBoV) is a newly discovered parvovirus associated with acute respiratory tract illness (ARTI) and gastrointestinal illness. Our study is the first to analyze the characteristics of HBoV-positive samples from ARTI patients with a wide age distribution from Guangzhou, southern China.

Methods: Throat swabs (n=2811) were collected and analyzed from children and adults with ARTI over a 13-month period. The HBoV complete genome from a 60 year-old female patient isolate was also determined.

Results: HBoV DNA was detected in 65/2811 (2.3%) samples, of which 61/1797 were from children (<18 years old) and 4/1014 from adults (≥18 years old). Seasonal peaks of 4.8% and 7.7% were detected in May and June, respectively. 28 of 65 (43.1%) HBoV-positive samples were co-detected with 11/16 other potential pathogens. *Mycoplasma pneumoniae* had the highest frequency of 16.9% (11/65). Upper and lower respiratory tract illness were common symptoms, with 19/65 (29.2%) patients diagnosed with pneumonia by chest radiography. All four adult patients had systemic influenza-like symptoms. Phylogenetic analysis of the complete genome revealed a close relationship with other HBoVs, and a more distant relationship with HBoV2 and HBoV3.

Conclusions: HBoV was detected from children and adults with ARTI from Guangzhou, southern China. Elderly people were also susceptive to HBoV. A single lineage of HBoV was detected among a wide age distribution of patients with ARTI.

Background

Respiratory tract infection etiology is complex and diverse, and new pathogens are continuously being reported. Over the past few years, several novel respiratory viruses including human metapneumovirus (hMPV) [1], severe acute respiratory syndrome (SARS) coronavirus [2], human coronavirus NL63 (HCoV-NL63) [3,4], and coronavirus HKU1 (HCoV-HKU1) [5-7] have been identified.

In 2005, Allander et al. [8] reported a previously undescribed human parvovirus, human bocavirus (HBoV) that belongs to the genus *Bocavirus*, in respiratory secretions of children with respiratory tract disease in Sweden. HBoV is a single-stranded deoxyribonucleic acid (DNA) virus with a small genome size of approximately 5.3 kilo-bases (kb), which has three open reading frames (ORF) encoding two non-structural proteins NS1 and NP1, and the two structural proteins VP1 and VP2. VP1 and VP2 are located within the same ORF but have different initiator codon positions [8].

Subsequently, HBoV was reported in respiratory samples from different countries and regions worldwide [9-14], where HBoV was detected in 1.5%-8.3% of respiratory samples from individuals with acute respiratory tract illness (ARTI), especially young children and infants. The virus was also found in stool samples from patients with gastrointestinal illness [15-22]. These reports suggest that HBoV might be associated with upper and lower respiratory disease and gastrointestinal illness throughout the world. In 2009, two viruses closely related to HBoV, named HBoV2 [23] and HBoV3 [24], were found in stool samples, and suggested HBoV diversity.

HBoV infection has recently attracted increasing attention all over the world. However, the incidence and clinical presentation of this infection varies widely, and often

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European Journal of Medicinal Chemistry 46 (2011) 52–57

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Discovery of highly potent agents against influenza A virus

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ARTICLE INFO

Article history:
Received 10 August 2010
Received in revised form
8 October 2010
Accepted 9 October 2010
Available online 16 October 2010

Keywords:
Hit-to-lead optimization
Influenza A virus
SAR study

ABSTRACT

We previously reported several new M2 inhibitors as active as amantadine against influenza A virus and validated by three types of *in vitro* assays. Herein, we further modified one of the most potent hits in a viral inhibition assay and conducted structure–activity relationship studies on this scaffold. As a result, compound **8e** was identified to be the most potent inhibitor against wild-type influenza A virus, being nearly 240-fold more active than amantadine.

1. Introduction

Influenza is always a major worldwide health threat to humankind. In the face of the recent outbreak of highly pathogenic avian influenza (H5N1) in Southeast Asia and H1N1 influenza (swine flu) around the world [1,2], there is a great need for anti-influenza therapeutics. However, very few effective drugs are available to combat the influenza virus. Amantadine (**1**) and rimantadine (**2**) (Fig. 1), which target the influenza A virus M2 (matrix-2 protein) ion channel, have long been available for both the prophylaxis and therapy of influenza A viral infections, but their use has been limited because of the rapid emergence of drug resistance and, particularly for amantadine, the occurrence of central nervous system (CNS) side effects [3]. Over the past decade, nearly all reported M2 inhibitors have been amantadine derivatives, such as compound **3** (Fig. 1) [4–9]. Compound **4** is one of the very few examples of nonadamantane-based M2 inhibitors that have been reported [10–12]. Therefore, there is an increasingly urgent need to discover new types of M2 inhibitors for the development of new anti-influenza drugs.

Recently, we reported the identification of several new hits as M2 inhibitors through the focused screening of a small primary amine library [13]. The hits were as active as amantadine against wild-type influenza A virus as determined by three kinds of assays, including cell-based, viral inhibition and patch clamp assays. Among them, compound **5** was the most potent inhibitor and was three times more active than amantadine for viral inhibition ($IC_{50} = 1.363$ μM vs. 5.960 μM).

Encouraged by these results, we decided to modify the hit to further increase its potency. By keeping the scaffold constant and modifying the amino functionality, 14 analogs were made and evaluated for viral inhibition, as assessed by A/WS/33 (H1N1, amantadine resistant) and A/Hong Kong/8/68 (H3N2, amantadine sensitive) viruses [14,15]. Most of the compounds in this study exhibited antiviral inhibition as good as amantadine, and compound **8e** was identified to be the most potent; it was nearly 240-fold more potent than amantadine.

2. Chemistry

The synthesis of (1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheylamine amido derivatives **7a**–**7g** is shown in Scheme 1. Initially, commercially available compound **5** was *N*-acylated with methyl chloroformate or acetyl chloride to give **6a** and **6b**, which were then reduced with $LiAlH_4$. Following reduction, salification with HCl/CH_3OH gave **7a** and **7b** [16]. On the other hand, compounds **7c**–**g** were obtained through a one-pot synthesis in which compound **5** was first coupled with different aldehydes or ketones. This synthesis was then followed by hydrogenation with $NaBH(OAc)_3$ in methanol [17] and finally treated with HCl/CH_3OH to provide salts **7c**–**g**.

Abbreviations: M2, matrix-2 protein; SAR, structure–activity relationship.

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doi:10.1016/j.ejmech.2010.10.010

Respiratory Physiology & Neurobiology 179 (2011) 219–226

Contents lists available at SciVerse ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Effects of dead space loading on neuro-muscular and neuro-ventilatory coupling of the respiratory system during exercise in healthy adults: Implications for dyspnea and exercise tolerance

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ARTICLE INFO

Article history:
Accepted 17 August 2011

Keywords:
Dyspnea
Exercise
Diaphragm
Electromyography
Dead space

ABSTRACT

We examined the effects of dead space loading (DSL) on ventilation ($\dot{V}E$), neural respiratory drive (EMGdi%max, diaphragm EMG expressed as a % of maximal EMGdi), contractile respiratory muscle effort (Pes,tidal%P_{Imax}, tidal esophageal pressure swing expressed as a % of maximal inspiratory Pes) and exertional dyspnea intensity ratings in 11 healthy adults with normal spirometry. Subjects completed, in random order, symptom-limited incremental cycle exercise tests under control (CTRL) and DSL (500 ml) conditions. Compared with CTRL, DSL decreased exercise tolerance by 20–25%; increased exertional dyspnea intensity ratings in direct proportion to concurrent increases in EMGdi%max, Pes,tidal%P_{Imax} and $\dot{V}E$; and had little/no effect on the inter-relationships between EMGdi%max, Pes,tidal%P_{Imax} and $\dot{V}E$ during exercise. In conclusion, DSL was associated with an earlier onset of intolerable dyspnea; however, neuromuscular and neuro-ventilatory coupling of the respiratory system remained relatively preserved during exercise in the presence of an increased external dead space. Under these circumstances, DSL-induced increases in exertional dyspnea intensity ratings reflected, at least in part, the awareness of increased neural respiratory drive, contractile respiratory muscle effort and ventilatory output.

1. Introduction

Dyspnea (respiratory discomfort) on exertion is the dominant symptom of patients with chronic cardiorespiratory disease (e.g., obstructive and restrictive pulmonary disease, pulmonary vascular disease, congestive heart failure) and contributes importantly to exercise intolerance and an impoverished health-related quality-of-life in these patients (O'Donnell et al., 2006, 2007, 2009; Sajkov et al., 2010). There is increasing evidence that troublesome activity-related dyspnea and progressive reductions in aerobic working capacity also arises as a consequence of normative aging (Ofir et al., 2008; Jensen et al., 2009). In all cases, the increase in dyspnea and decrease in exercise tolerance is associated with concurrent increases in central (neural) respiratory motor command output, primarily as a result of increases in physiological dead space and/or ventilation-perfusion mismatching (Ofir et al., 2008; Jensen et al., 2009; O'Donnell et al., 2009; Van der Plas et al., 2010). However, the specific inter-relationships between increased neural respiratory drive, exertional dyspnea and activity-limitation are not completely understood and represent the primary focus of this study.

Increases in central motor command output to the respiratory muscles have the potential to curtail exercise performance by (i) accelerating the rise in exertional dyspnea intensity ratings thereby leading to an earlier onset of intolerable dyspnea, (ii) hastening or shortening the time to reach a critical mechanical constraint on thoracic volume displacement with attendant uncoupling of the inter-relationships between neural respiratory drive, contractile respiratory muscle pressure/force generation and ventilatory output or (iii) a combination of these factors. The purpose of the present study, therefore, was to examine the acute effects of dead space loading (DSL) on ventilation ($\dot{V}E$), breathing pattern, multipair esophageal electrode catheter-derived measures of the diaphragm electromyogram (EMGdi), esophageal pressure (Pes)-derived measures of contractile respiratory muscle effort, exertional dyspnea intensity ratings and exercise performance in healthy, older adults. Briefly, increased external physiological dead space stresses the ventilatory control system such that a greater $\dot{V}E$ is required to affect the same arterial P_{CO_2} (Pa_{CO_2}) and H^+ regulation at any given metabolic rate, in accordance with the alveolar gas equation for CO_2: $\dot{V}E = (\dot{V}_{CO_2} \times 863)/(Pa_{CO_2} \times [1 - VD/VT])$, where $\dot{V}_{CO_2}$ and VD/VT represent the metabolic rate of CO_2 production and

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doi:10.1016/j.resp.2011.08.009

Vaccine 29 (2011) 3837–3841

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Short communication

Epidemiology of adenovirus type 5 neutralizing antibodies in healthy people and AIDS patients in Guangzhou, southern China

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ARTICLE INFO

Article history:
Received 25 October 2010
Received in revised form 10 March 2011
Accepted 12 March 2011
Available online 27 March 2011

Keywords:
Adenovirus
Antibody
AIDS
HIV,vaccine

ABSTRACT

Recombinant adenovirus serotype 5 (Ad5) viruses have been extensively explored as vectors for vaccination or gene therapy. However, one major obstacle to their clinical application is the high prevalence of preexisting anti-Ad5 immunity resulting from natural infection. It has been reported that there are geographic variations in the prevalence of natural adenovirus infection. In the present study, we investigated the seroprevalence of Ad5 in Guangzhou, southern China by measuring the Ad5 neutralizing antibodies in blood samples collected from several sites. The seroprevalence was 77.34% in the general healthy population. The seroprevalence and antibody titers increased with age, with the older population (41-72 years old) having the highest seropositivity (84.8%) and percentage (54.4%) of high Ad5 neutralizing antibody titers (>1000). The dynamics of Ad5 neutralizing antibodies were stable and persistent over the course of eight months. Furthermore, the seroprevalence of Ad5 in the HIV-infected AIDS patients was investigated and there was no significant difference from the general healthy population. Our survey provides useful insights for the future development of Ad5-based vaccination and gene therapy.

1. Background

Recombinant adenoviruses, especially replication-defective adenovirus serotype 5 (Ad5), have been extensively explored as vectors for gene therapy or vaccine against human immunodeficiency virus (HIV), hepatitis virus, influenza virus, other infectious diseases and cancers [1–4]. However, the practical application of Ad5 has been potentially limited by the high prevalence of preexisting anti-Ad5 immunity. Both preclinical animal models and human clinical trials have demonstrated that preexisting anti-Ad5 immunity can significantly reduce the expression level of transgene of Ad5-based vectors [5–7], thus impair its ability to generate immune responses against the target antigens delivered by Ad5 vectors. Therefore, it is necessary to determine the Ad5 prevalence in local areas prior to the administration of Ad5 vector-based products.

It has been reported that there are geographic variations in the prevalence of natural adenovirus infection. For example, approximately 40%-69% of adult populations in America [8,14], and more than 93% of pediatric populations in Sub-Saharan Africa [9] are Ad5-seropositive. However, there is no report on the seroprevalence of Ad5 in a Chinese population. Therefore, in the present study we investigated the seroprevalence of Ad5 in the general healthy population and in HIV-infected AIDS patients in Guangzhou southern China.

2. Materials and methods

2.1. Human samples

Serum samples were isolated from 278 healthy participants and 32 HIV-infected AIDS patients in Guangzhou, Guangdong province, southern China. The participants' ages ranged from 6 to 72 years old, and the sex ratio was approximately 1:1 (male:female = 51.8%:48.2%). The samples from healthy participants were collected from the Guangzhou Center for Disease Control and Prevention (CDC), Guangzhou Children's Hospital, and Guangzhou Institute of Biomedicine and Health (GIBH), and the samples from HIV-infected AIDS patients were collected from Guangzhou No. 8 People's Hospital (Guangzhou Hospital for Infectious Diseases). The research involving human participants in our study was approval by the Ethics Committee of Guangzhou Institute of Biomedical and Health (GIBH), Chinese Academy of Sciences,

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doi:10.1016/j.vaccine.2011.03.042

Clin Exp Med (2011) 11:19–24
DOI 10.1007/s10238-010-0106-5

ORIGINAL ARTICLE

Establishment of airway eosinophilic bronchitis mouse model without hyperresponsiveness by ovalbumin

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Received: 7 January 2010 / Accepted: 19 July 2010 / Published online: 5 August 2010

Abstract Eosinophilic bronchitis (EB) is a useful tool for studying airway hyperresponsiveness (AHR), but an exact EB animal model is lacking. Our objective was to establish an EB mouse model using ovalbumin (OVA). Mice were divided into asthma, normal saline (NS) control and three model groups. Asthma mice were challenged intranasally with 200 μg OVA. Model groups were challenged with one of the three OVA doses (10, 20 or 100 μg), and NS control mice received normal saline. Changes in lung resistance (R_L), serum OVA-specific immunoglobulin-E (IgE) and differential inflammatory cell counts in bronchoalveolar lavage fluid (BALF) were determined after exposure to increasing doses of methacholine (MCh). Lung histological sections were examined for inflammatory infiltration. R_L in the 10-μg OVA-challenged model group was not significantly different compared with the NS group at any MCh concentration but was significantly different compared with the asthma group ($P < 0.01$). R_L in the other two model groups was intermediate between the asthma and NS groups. Serum OVA-specific IgE and eosinophils in BALF were increased significantly in all model and asthma groups compared with the NS group, but no significant differences were observed between model and asthma groups. Inflammatory cells were seen around bronchioles and capillaries in model and asthma groups but not the NS group. A mouse model of EB without AHR can be established by 10 μg OVA challenge and provides a useful tool for studying AHR mechanisms.

Keywords Airway eosinophilia · Asthma · Mouse model · Airway responsiveness · Eosinophilic bronchitis

Introduction

Asthma is a serious public health problem worldwide, affecting people of all ages. When uncontrolled, asthma can severely limit activities of daily living and can even be fatal [1]. Airway hyperresponsiveness (AHR) and airway inflammation(AI) are two hallmarks of asthma. Measurement of bronchial responsiveness is widely used to diagnose and monitor asthma [2]. In last two decades, most studies have investigated the relationship between AI and AHR. However, researchers have found that AHR is associated not only with inflammation, but also with airway repair, excessive contraction of airway smooth muscle, uncoupling of airway contraction, thickening of airway wall and activation of sensory nerves [3]. Despite intensive research, the complex mechanisms of AHR pathogenesis remain unclear.

Nonasthmatic eosinophilic bronchitis (EB) was first identified by Gibson and coworkers as a major cause of chronic cough [4]. They described a group of patients with chronic cough, sputum evidence of eosinophils, but normal spirometry, no evidence of AHR and normal PEF variability. The features of this condition were distinct from asthma; thus, it was named EB. Similar to asthma, EB is associated with exposure to an occupational sensitizer and commonly inhaled allergens [5–10]. EB is generally defined as an allergic disease because most EB patients

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AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 28, Number 4, 2012

DOI: 10.1089/aid.2011.0061

Enhancement of Gag-Specific But Reduction of Env- and Pol-Specific CD8$^+$ T Cell Responses by Simian Immunodeficiency Virus Nonstructural Proteins In Mice

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Abstract

Accessory and regulatory proteins (nonstructural proteins) have received increasing attention as components in novel HIV/SIV vaccine design. However, the complicated interactions between nonstructural proteins and structural proteins remain poorly understood, especially their effects on immunogenicity. In this study, the immunogenicity of structural proteins in the presence and absence of nonstructural proteins was compared. First, a series of recombinant plasmids and adenoviral vectors carrying various SIVmac239 nonstructural and structural genes was constructed. Then mice were primed with DNA plasmids and boosted with corresponding Ad5 vectors of different combinations, and the resulting immune responses were measured. Our results demonstrated that when the individual Gag, Pol, or Env gene products were coimmunized with the whole repertoire of nonstructural proteins, the Gag-specific CD8$^+$ T response was greatly enhanced, while the Env- and Pol-specific CD8$^+$ T responses were significantly reduced. The same pattern was not observed in CD4$^+$ T cell responses. Antibody responses against both the Gag and Env proteins were elicited more effectively when these structural antigens were immunized together with nonstructural antigens. These findings may provide helpful insights into the development of novel HIV/SIV vaccines.

Introduction

To date, hundreds of millions of individuals have been infected by the HIV-1 virus. Development of an effective vaccine is fraught with difficulty, and thus far, a satisfactory outcome has yet to be achieved.[1,2] Currently, even the precise correlation between the immune response and viral protection remains unknown.[3–5] In addition to the classical vaccine, based on neutralizing antibodies, T cell-based vaccines were considered very promising,[6–8] though Merck's announcement of the frustrating failure of their STEP trial has led to the T cell vaccine being contested.[9,10] However, the reasons for the vaccine's failure appear to be very complicated,[11–13] and the limited breadth of the induced immune response is thought to be one potential explanation.[14,15] Encouragingly, it has been reported that several novel vaccine regimens that were targeted to enhance and broaden T cell responses could control simian immunodeficiency virus (SIV) viral replication at the peak and chronic phases of infection.[8,16–18]

In addition to structural proteins (Gag, Pol, and Env), which have traditionally been the major targets in HIV/SIV vaccine design, the genome of HIV/SIV contains genes encoding regulatory (Tat and Rev) and accessory proteins (Nef, Vif, Vpr, and Vpu/Vpx). Tat and Rev are essential for viral replication.[19–21] Because the mRNAs of Tat, Rev, and Nef are fully spliced and transported from the nucleus to the cytoplasm, these are the first proteins expressed during the life cycle of HIV/SIV. Although Nef, Vif, Vpr, Vpx and Vpu are dispensable for viral growth,[22,23] these accessory proteins are necessary for viral pathogenesis and play very important roles in maintaining high levels of viral replication and carrying out other functions.[24] Given the importance of regulatory and accessory proteins in viral survival, these proteins are promising targets for vaccines against HIV/SIV. Moreover, by screening HIV-1 patients with overlapping viral peptide pools, it has been shown that all regulatory and accessory proteins serve as targets for cytotoxic T lymphocytes (CTL).[25–31] Therefore, to broaden T cell responses, increasing

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PLoS one

Epigenetic Dysregulation in Mesenchymal Stem Cell Aging and Spontaneous Differentiation

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Abstract

Background: Mesenchymal stem cells (MSCs) hold great promise for the treatment of difficult diseases. As MSCs represent a rare cell population, *ex vivo* expansion of MSCs is indispensable to obtain sufficient amounts of cells for therapies and tissue engineering. However, spontaneous differentiation and aging of MSCs occur during expansion and the molecular mechanisms involved have been poorly understood.

Methodology/Principal Findings: Human MSCs in early and late passages were examined for their expression of genes involved in osteogenesis to determine their spontaneous differentiation towards osteoblasts *in vitro*, and of genes involved in self-renewal and proliferation for multipotent differentiation potential. In parallel, promoter DNA methylation and hostone H3 acetylation levels were determined. We found that MSCs underwent aging and spontaneous osteogenic differentiation upon regular culture expansion, with progressive downregulation of TERT and upregulation of osteogenic genes such as Runx2 and ALP. Meanwhile, the expression of genes associated with stem cell self-renewal such as Oct4 and Sox2 declined markedly. Notably, the altered expression of these genes were closely associated with epigenetic dysregulation of histone H3 acetylation in K9 and K14, but not with methylation of CpG islands in the promoter regions of most of these genes. bFGF promoted MSC proliferation and suppressed its spontaneous osteogenic differentiation, with corresponding changes in histone H3 acetylation in TERT, Oct4, Sox2, Runx2 and ALP genes.

Conclusions/Significance: Our results indicate that histone H3 acetylation, which can be modulated by extrinsic signals, plays a key role in regulating MSC aging and differentiation.

Citation: Li Z, Liu C, Xie Z, Song P, Zhao RCH, et al. (2011) Epigenetic Dysregulation in Mesenchymal Stem Cell Aging and Spontaneous Differentiation. PLoS ONE 6(6): e20526. doi:10.1371/journal.pone.0020526

Editor: Eliana Saul Furquim Werneck Abdelhay, Instituto Nacional de Cancer, Brazil

Received March 9, 2011; **Accepted** May 2, 2011; **Published** June 9, 2011

Funding: This work was supported by grants from the Natural Science Foundation of China (No. 30871273, 30971496, U1032003) to Y.W. and by the National Basic Research Program (No. 2009CB522108) to L.Z.G. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Mesenchymal stem cells (MSCs) are self-renewing and expandable stem cells [1,2,3]. In order to compare and contrast study outcomes from different research groups, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a minimal criterion to define human MSCs. First, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSCs must be lineage-negative and express CD105, CD73 and CD90. Third, MSCs must differentiate to at least osteoblasts, adipocytes and chondroblasts *ex vivo* [3].

Increasing evidence has suggested profound therapeutic potential of MSCs for a variety of diseases such as myocardial infarction [4,5,6,7], neural diseases [8,9] strokes [10], and wound healing [11,12]. Moreover, allogeneic MSCs have shown low immunogenicity and immunosuppressive properties [13,14,15]. Due to encouraging preclinical results, numerous clinical trials for a variety of diseases are underway [16,17,18,19,20].

MSCs represent as a rare cell population in the bone marrow (BM) and other tissues. BM is the major source of MSCs, where they represent only approximately 0.001% to 0.01% of the nucleated cells, about 10-fold less abundant than hematopoietic stem cells (HSCs). Therefore, *ex vivo* expansion of MSCs is an indispensable procedure to obtain sufficient amounts of cells for MSC-based therapies and tissue engineering. MSCs are capable of proliferating in culture [1,2], and they are genetically stable when undergoing limited *ex vivo* expansion [21]. However, recent studies suggest MSCs age rapidly in culture and undergo considerable property changes. This has raised concerns over the effect and safety of MSC-based therapies [22,23]. More importantly, the molecular mechanisms underlying phonotypical changes of MSCs during culture expansion are unclear.

In this study, we found that MSCs underwent considerable epigenetic and gene expressional alterations during culture expansion, even though the morphological changes are modest. The expression of osteogenic genes increased progressively with

Protein Expression and Purification 80 (2011) 68–73

Contents lists available at ScienceDirect

Protein Expression and Purification

journal homepage: www.elsevier.com/locate/yprep

Expression, purification, and refolding of active human and mouse secreted group IIE phospholipase A_2

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ARTICLE INFO

Article history:
Received 25 April 2011
and in revised form 3 May 2011
Available online 14 May 2011

Keywords:
sPLA2 group II E
Inclusion body
Refolding
Phospholipid hydrolysis

ABSTRACT

Secreted phospholipase A_2s form a large family of proteins involved in diverse biological and pathophysiological processes. Group IIE secreted phospholipase A_2 ($sPLA_2$-IIE) is one of the latest discovered members of this family. Previous studies revealed that the expression profile of $sPLA_2$-IIE was restricted to a few tissue types including brain, heart, lung and placenta compared to the broad expression profile of other isoforms. Accumulating evidence suggests that $sPLA_2$-IIE might play a pivotal role in the progression of inflammatory processes. However, functional study of $sPLA_2$-IIE was hindered by the low yield of soluble expressed protein. In this study, we have expressed human and mouse $sPLA_2$-IIE in *Escherichia coli* in the form of inclusion bodies. The inclusion bodies were dissolved, purified and refolded in a stepwise dialysis approach and further purified. We obtained soluble and active proteins for human and mouse $sPLA_2$-IIE with a final yield of 1.1 and 1.2 mg/500 mL bacterial culture, respectively. The refolded $sPLA_2$-IIEs exhibited similar calcium and pH dependence of their enzymatic activity with those expressed in COS cells. This protein expression and purification protocol will facilitate the further structural and functional studies of human and mouse $sPLA_2$-IIEs.

Introduction

Phospholipase A_2s (PLA_2 EC3.1.1.4) comprise a superfamily of enzymes that hydrolyze the *sn*-2 ester of glycerophospholipids and release free fatty acid and lysophospholipid [1,2]. PLA_2s play crucial roles in a wide variety of biological processes, including phospholipid digestion, metabolism, remodeling of cell membranes, signal transduction and host defense [3]. Apart from these physiological functions, PLA_2s also exhibit diverse and potent biological actions through their participation in various pathophysiological processes with the production of lipid derivatives; such as prostaglandins, leukotrienes, thromboxanes and platelet-activating factors [3]. PLA_2s have been divided into three major classes according to their catalytic mechanism, functional and structural features: secreted PLA_2s ($sPLA_2$), Ca^{2+}-dependent cytosolic PLA_2s and Ca^{2+}-independent cytosolic PLA_2s [4].

To date, 10 mammalian $sPLA_2$s have been identified and characterized [5]. Many of them are involved in the host defense against bacterial infections [6,7], the biosynthesis of eicosanoids during inflammation [8,9], and even in the process of atherosclerosis [10,11] and cancer [12–14]. $sPLA_2$s have several common features including a low molecular weight (14–18 kDa), 6–8 disulfide bonds, the absolute requirement of millimolar concentration of Ca^{2+} for catalytic activity and a broad specificity for phospholipids with different polar head groups and fatty acid chains [15,16]. In addition, with the discovery of several $sPLA_2$ receptor proteins, accumulating evidence shows that, other than their lipolytic enzymatic activity, $sPLA_2$s may also function by binding to their cellular target proteins [5]. It has been reported that, the expression of Group IIA secreted phospholipase A_2 ($sPLA_2$-IIA), the most widely studied $sPLA_2$ isoform, is dramatically enhanced at various inflamed sites and upregulated in various cells and tissues in response to proinflammatory stimuli [17–19]. This suggests that $sPLA_2$-IIA may play a pivotal role in inflammatory responses. However, the inbred C57BL6/J mice in which the $sPLA_2$-IIA gene is naturally inactivated [13,20] also developed arthritis in the antigen-induced model similar to the $sPLA_2$-IIA-expressing mice strain [21,22], implying that other isoforms besides $sPLA_2$-IIA might play compensatory roles.

The expression profiles of $sPLA_2$ isoforms are diverse. For example, human $sPLA_2$-IIA was detected broadly in multiple tissues

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doi:10.1016/j.pep.2011.05.002

Mol Biol Rep (2011) 38:1457–1462
DOI 10.1007/s11033-010-0251-7

Golgi phosphoprotein 2 (GOLPH2/GP73/GOLM1) interacts with secretory clusterin

Yan Zhou · Leike Li · Longbo Hu · Tao Peng

Received: 2 March 2010 / Accepted: 2 September 2010 / Published online: 15 September 2010

Abstract Golgi phosphoprotein 2 (GOLPH2/GP73/GOLM1), a type-II Golgi transmembrane protein of unknown function, is up-regulated in many cancers. Its Golgi luminal domain is potentially the major functional domain. The goal of this study is to identify the proteins interacting with GOLPH2. Using secretory GOLPH2 (sGOLPH2, amino acid residues 55–401) as bait, secretory clusterin (sCLU) was identified as one interacting candidate by yeast two-hybrid screening, and the coiled-coil domain of GOLPH2 was found to be sufficient for interaction with sCLU. The interaction between GOLPH2 and sCLU was confirmed intracellularly and extracellularly. The intracellular co-localization of GOLPH2 and sCLU in Golgi was also shown. These results can help in understanding the biological and pathological significance of GOLPH2.

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Keywords GOLPH2 · GP73 · Clusterin · Protein interaction · Coiled-coil domain

Introduction

Golgi phosphoprotein 2 (GOLPH2), also known as Golgi membrane protein1 (Golm1) or GP73, is widely expressed in normal epithelial cells of numerous tissues, especially in the gut, prostate, kidneys, lungs and within the central nervous system [1, 2]. In normal livers, GOLPH2 is constitutively expressed in biliary epithelial cells but not in hepatocytes [3]. However, markedly elevated GOLPH2 expression was observed in liver diseases [3]. Up-regulated GOLPH2 was also observed in certain cancers such as hepatocellular carcinoma (HCC), prostate cancer, and renal cell cancer [1, 4, 5]. The fact that GOLPH2 is expressed in both normal and diseased human tissues of the epithelial lineage implies that it might play an essential role in epithelial cells and carcinogenesis.

GOLPH2 contains a short N-terminal cytoplasmic domain, followed by a transmembrane domain (TMD) and a larger C-terminal domain facing the Golgi lumen. Being a type II transmembrane protein, its C-terminal ectodomain faces the Golgi lumen. Within this luminal region is a coiled-coil domain and an acid tail capable of mediating protein–protein interactions [3]. A proprotein convertase (PC) recognition site in the C-terminal ectodomain allows for PC-mediated cleavage (Fig. 1a), resulting in the secretion of GOLPH2 (sGOLPH2) from the hepatocytes [6].

Phenotypic analysis of a transgenic mouse model with C-terminally truncated GOLPH2 indicated that the lack of full-length GOLPH2 resulted in decreased survival and severe epithelial abnormalities of the kidney and the liver

Journal of Surgical Oncology 2011;104:598–603

Heat Shock Protein-60 Expression was Significantly Correlated With the Prognosis of Lung Adenocarcinoma

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Background: The purpose of this study was to investigate the role of heat shock protein 60 (HSP60) in the clinical pathology of lung adenocarcinoma, and to explore whether the expression of HSP60 can act as an independent predictor for tumor relapse and prognosis after radical resection of lung adenocarcinoma.

Methods: Paraffin sections of lung adenocarcinoma tumor tissues were collected from 103 patients. Using immunohistochemistry, the expression levels of HSP60 in lung adenocarcinoma were detected. The correlations between HSP60 expression and clinicopathological parameters as well as prognosis were statistically analyzed.

Results: Of the 103 specimens, 70 cases (68.0%) showed a strongly positive expression of HSP60, five cases (4.8%) showed a negative expression, and 28 cases (27.2%) showed a weakly positive expression. The level of HSP60 expression was significantly correlated with TNM stage of the tumor ($P = 0.015$), and Eastern Cooperative Oncology Group (ECOG) performance status($P = 0.027$). Multivariate statistical analysis showed that patient age, pathological T stage, N stage, and HSP60 expression were independent prognostic influence on disease-free survival ($P = 0.008$, 0.011, 0.010, and <0.001, respectively).

Conclusions: HSP60 may be a good biomarker to be applied in clinic to predict the prognosis of patients with lung adenocarcinoma
J. Surg. Oncol. 2011;104:598–603.

KEY WORDS: lung adenocarcinoma; heat shock protein 60; immunohistochemistry

INTRODUCTION

In both developed and developing countries, lung cancer incidence has been rising. It has become one of the common malignant tumors for humans and is now the leading cause of mortality worldwide [1]. Lung adenocarcinoma is one common type of non-small cell lung cancer (NSCLC), and is also the pathological type with the highest incidence in non-smoking patients. In recent years, the incidence of lung adenocarcinoma has increased significantly. Because of early metastasis in blood and relapse associated with lung adenocarcinoma, its prognosis is relatively poor [2,3]. With early stage of NSCLC, patients can be cured via surgical treatment and the 5-year survival rate can reach 70% [4]. However, for most patients with NSCLC, treatment is usually not available at the early stages. Even after the combination treatment of surgery, chemotherapy, radiotherapy, and targeted drug therapy is given, most patients die of lung cancer relapse or metastasis.

The relapse and metastasis common in these lung cancer patients may be related to the micro-metastasis of tumor cells, where such micro-metastasis disease lesions might have already occurred even before the surgery [5,6]. Therefore, the existence of a relevant clinical indicator that can accurately predict the relapse, metastasis, and prognosis of lung cancer will be very beneficial for clinicians in their decisions regarding appropriate treatments. Currently, many clinical and fundamental research projects have found that some genes or proteins, such as vascular endothelial growth factor (VEGF) [7], carcinoembryonic antigen (CEA) [8,9], cytokeratin 19 Fragment (CYFRA 21-1) [9], and S100A11 [10] were associated with the prognosis of lung cancer prognosis. Moreover, some scholars have found that the detection of mRNA and proteins of certain genes can predict the prognosis of lung adenocarcinoma [11–14]. However, these indicators of detection are yet to accurately predict the relapse, metastasis, and prognosis of lung cancer. As such, finding relevant laboratory indicators that can accurately predict the relapse and metastasis of lung cancers has become the focus of many researchers.

Heat shock protein 60 (HSP60), an important member in the heat shock protein family, is a highly conserved protein that is widespread in the biosphere. Previous studies have indicated that the expression of HSP60 in a variety of tumors is related to the malignant degree of the tumor and the clinical prognosis [15–21]. For lung cancer, it has also been reported that the expression of HSP60 was related to the initiation of lung cancer [22,23]. However, in previous studies, the association between HSP60 expression and prognosis of patients

Grant sponsor: Science and Technology Planning Project of Guangdong Province, China; Grant numbers: 2007B031515017; 2008A030201024; Grant sponsor: Science and Technology Planning Project of Guangzhou, China; Grant numbers: 2007Z1-E0111. 2007Z3-E0261.

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Received 26 April 2011; Accepted 20 May 2011

DOI 10.1002/jso.21992

Published online 13 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

Hepatocyte Nuclear Factor-4 Alpha Regulates Liver Triglyceride Metabolism in Part Through Secreted Phospholipase A_2 GXIIB

Min Guan, Linbing Qu, Wenjuan Tan, Ling Chen, and Chi-Wai Wong

Hepatocyte nuclear factor-4 alpha (HNF-4α) is an important transcription factor governing the expression of genes involved in multiple metabolic pathways. Secreted phospholipase A_2 GXIIB (PLA_2GXIIB) is an atypical member of a class of secreted phospholipases A_2. We establish in this study that PLA_2GXIIB is an HNF-4α target gene. We demonstrate that HNF-4α binds to a response element on the PLA_2GXIIB promoter. Moreover, HNF-4α agonists induce PLA_2GXIIB expression in human hepatocarcinoma cells. Importantly, *PLA_2GXIIB*-null mice accumulate triglyceride, cholesterol, and fatty acids in the liver and develop severe hepatosteatosis resembling some of the phenotypes of liver-specific *HNF-4α*–null mice. These defects are in part due to compromised hepatic very low-density lipoprotein secretion. Finally, adenovirus-mediated overexpression of HNF-4α elevates serum triglyceride level in wild-type but not *PLA_2GXIIB*-null mice. *Conclusion:* Collectively, these evidences suggest that HNF-4α is a key physiological PLA_2GXIIB transcriptional regulator and that PLA_2GXIIB is a novel mediator of triglyceride metabolism in the liver. (HEPATOLOGY 2011;53:458-466)

Hepatocyte nuclear factor-4 alpha (HNF-4α) was initially identified as a transcriptional factor required for liver-specific gene expression.[1] Coenzyme A (CoA) derivatives of certain fatty acids can bind to the ligand-binding domain of HNF-4α and activate the receptor,[2] suggesting that HNF-4α activity is subjected to modulation by metabolic and nutritional signals. HNF-4α regulates the expression of gluconeogenic genes, including phosphoenolpyruvate carboxykinase (PEPCK)[3] and glucose-6-phosphatase (G6P),[4] through binding to hormone response elements on their promoters and recruiting coactivators such as peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) to their promoters.[5] In addition, HNF-4α and PGC-1α regulate the expression of lipoproteins and packaging enzymes such as microsomal triglyceride transfer protein (MTP) that are involved in very low-density lipoprotein (VLDL) secretion. Significantly, liver-specific HNF-4α-null (*HNF4αLivKO*) mice suffer from severe defects in lipid homeostasis with hepatosteatosis and reduced serum triglyceride (TG) and cholesterol levels.[6]

Phospholipases A_2 are enzymes that catalyze the hydrolysis of glycerophospholipids at the S_n2 position to release free fatty acids such as arachidonic acid and lysophospholipids that are precursors of signaling molecules.[7] In particular, arachidonic acid and its metabolites leukotrienes and prostaglandins are key inflammatory regulators. On the basis of their protein structures and biochemical properties, the superfamily of PLA_2 can be divided into five principal kinds of enzyme: cytosolic PLA_2s (cPLA_2s), Ca^{2+}-independent PLA_2s (iPLA_2s), lysosomal PLA_2s, platelet activating factor acetylhydrolases, and secreted PLA_2s (sPLA_2s).

Secreted PLA_2s have relatively low molecular masses of 14-19 kDa, a large number of disulfides, and similar Ca^{2+}-dependent catalytic mechanism. Mammalian sPLA_2s include *GIB*, *GIIA*, *GIIC*, *GIID*, *GIIE*, *GIIF*, *GIII*, *GV*, *GX*, *GXIA*, *GXIB*, *GXIIA*, and *GXIIB*.

Abbreviations: Apo, apolipoprotein; bp, base pair; CoA, coenzyme A; EMSA, electrophoresis mobility shift assay; G6P, glucose-6-phosphatase; HNF-4α, hepatocyte nuclear factor-4 alpha; kb, kilobase; mRNA, messenger RNA; MTP, microsomal triglyceride transfer protein; PCR, polymerase chain reaction; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; PLA_2, phospholipase A_2; TG, triglyceride; VLDL, very low-density lipoprotein.

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View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.24066

Potential conflict of interest: Nothing to report.

Additional Supporting Information may be found in the online version of this article.

Jpn. J. Infect. Dis., 64, 190-194, 2011

Original Article

Immunogenicity and Safety of a China-Made Monovalent Pandemic (H1N1) 2009 Influenza A Vaccine in Healthcare Workers in Guangzhou, China

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(Received November 26, 2010. Accepted March 11, 2011)

SUMMARY: Because healthcare workers played an important role in the battle against novel pandemic (H1N1) 2009 influenza, a clinical study was conducted to examine the immunogenicity and safety of a single dose of a China-made monovalent, split-virus, pandemic (H1N1) 2009 influenza vaccine in this special high-risk population. Healthcare workers in the First Affiliated Hospital of Guangzhou Medical College who received the pandemic (H1N1) 2009 influenza vaccine were prospectively enrolled. Antibody titers were measured at enrollment and 14 days later using hemagglutination-inhibition (HI) and microneutralization assays. Adverse events were recorded within 7 days and 6 months after vaccination. Double sera were provided by 51 of 65 enrolled subjects. Postvaccination titers of 1:40 or more on HI assay were observed in 96% of recipients. Seroconversion or a significant increase in titer on HI assay occurred in 59% of subjects. The factor increase in GMTs was 4.3. The majority of complaints were mild to moderate in intensity. Although more than half of healthcare workers seemed immune to the pandemic (H1N1) influenza A virus before vaccination, most of the subjects still showed a fast, robust immune response to a single 15-μg dose of a monovalent, split-virus unadjuvanted pandemic H1N1 2009 influenza vaccine.

INTRODUCTION

In April 2009, the Centers for Disease Control and Prevention (CDC) in the United States (1) and the General Directorate of Epidemiology (GDE) in Mexico (2) identified several human cases of infection with pandemic (H1N1) 2009 influenza A virus (pandemic H1N1, also called novel H1N1, swine-origin influenza virus, or swine flu).

The first cases of pandemic H1N1 infection in China and Guangzhou were documented on May 10 and May 20, 2009, respectively. Pandemic H1N1 infection became prevalent beginning in September 2009 in Guangzhou (3), where influenza was uncommon during autumn and winter (4). It was deemed an unseasonal influenza virus in Guangzhou and worldwide because of its outbreak time in Mexico and the United States (1,2). The virus was subsequently determined to be a descendant of the 1918 pandemic strain (5), and was characterized by a unique triple reassortment of gene segments that had never before been identified in humans, pigs, or birds (6,7). There was concern that little protective immune memory exists in the general human population in China (8). The rapid spread of the virus worldwide in less than 2 months (9) led the WHO to raise the alert level of influenza pandemic from phase 3 to phase 6 (10).

Social intervention, i.e., contaminant policy or closure of schools, has been shown to be effective during the early period of a pandemic (11). However, it becomes useless once the influenza virus is prevalent in the community. An influenza pandemic will not wane until an immune barrier is set up in the majority of the public. Therefore, vaccination was considered to be the most effective weapon in the battle against the influenza pandemic and epidemic. The availability of safe and effective vaccines was critical for the prevention of pandemic H1N1 infection.

Healthcare workers are recommended targets for vaccination during epidemics and pandemics due to their special role in the battle against influenza (12). Vaccination can reduce the probability of nosocomial pandemic H1N1 infection caused by healthcare workers to a large degree (12). Inapparent infection, a critical and potential source of infection, has a high probability occur-

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RESPIRATORY RESEARCH

RESEARCH Open Access

Influence of degree of specific allergic sensitivity on severity of rhinitis and asthma in Chinese allergic patients

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China Alliance of Research on Respiratory Allergic Disease (CARRAD)

Abstract

Background: The association between sensitizations and severity of allergic diseases is controversial.

Objective: This study was to investigate the association between severity of asthma and rhinitis and degree of specific allergic sensitization in allergic patients in China.

Method: A cross-sectional survey was performed in 6304 patients with asthma and/or rhinitis from 4 regions of China. Patients completed a standardized questionnaire documenting their respiratory and allergic symptoms, their impact on sleep, daily activities, school and work. They also underwent skin prick tests with 13 common aeroallergens. Among the recruited subjects, 2268 provided blood samples for serum measurement of specific IgE (sIgE) against 16 common aeroallergens.

Results: Significantly higher percentage of patients with moderate-severe intermittent rhinitis were sensitized to outdoor allergens while percentage of patients sensitized to indoor allergens was increased with increasing severity of asthma. Moderate-severe intermittent rhinitis was associated with the skin wheal size and the level of sIgE to *Artemisia vulgaris* and *Ambrosia artemisifolia* ($p < 0.001$). Moderate-severe asthma was associated with increasing wheal size and sIgE response to *Dermatophagoides* (*D.*) *pteronyssinus* and *D. farinae* ($p < 0.001$). Moderate-severe rhinitis and asthma were also associated with increase in number of positive skin prick test and sIgE.

Conclusions: *Artemisia vulgaris* and *Ambrosia artemisifolia* sensitizations are associated with the severity of intermittent rhinitis and *D. pteronyssinus* and *D. farinae* sensitizations are associated with increasing severity of asthma in China. Increase in number of allergens the patients are sensitized to may also increase the severity of rhinitis and asthma.

Keywords: sensitization, aeroallergens, disease severity, allergic rhinitis, asthma, association.

Background

The prevalence of asthma and allergic rhinitis symptoms varies considerably across the world [1,2]. In China, the prevalence of allergic rhinoconjunctivitis symptoms varies from 8.7 to 24.1% documented by self-reported telephone interviews conducted between 2004 and 2005 in 11 cities [3]. The prevalence of respiratory allergy is increasing in China [3,4] and an international comparative study found that in the city of Guangzhou, the prevalence of asthma symptoms among children aged 13-14 years increased from 3.4% in 1995 to 4.8% in 2001 [4] and to 6.1% in 2009 (unpublished data).

Atopic sensitization is a risk factor for the development of upper and lower respiratory symptoms [5,6]. Exposure to allergens the patients are sensitized to may exacerbate symptoms of rhinitis and asthma by promoting airway inflammation, airflow limitation, and airway hyperreponsiveness (AHR). Sensitization to indoor allergens correlates well with indoor allergen exposure in pre-school and school-age children [7,8]. Furthermore, exposure and

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Ambrosia artemisifolia and moderate-severe intermittent rhinitis. Although we did not analyze the data by stratification of the patients with regions and seasons in this paper, we predict that these patients are mainly from the northern parts of China undergoing clinical sampling during the season from July to September [12]. One recently published study [11] demonstrated that sIgE levels to birch- and grass-pollen at baseline as well as during the pollen season were associated with seasonal symptom severity of rhinitis and use of rescue medications. In contrast, adult patients with seasonal allergic rhinitis have been investigated by several studies in this respect. Some investigators found a positive association between sIgE levels and clinical symptoms [29,30], although symptoms were also dependent on other factors, such as the ease of histamine release by basophils. Other studies did not find strong associations or reported inconsistent findings [31,32]. This inconsistency may be explained by differences in allergens, age or other characteristics of the patient populations studied. At least this seems to be the reason for a marked variability in the outcome of a variety of studies investigating the capacity to predict symptomatic allergy from sIgE levels in children [33]. We therefore assume that some of the above-mentioned differences among studies in respiratory allergies may be explained by the varying parameters of the allergens studied, the age of the patients and the measurements of clinical disease severity.

Surprisingly, we failed to find the relationship between HDM skin test size and specific IgE levels and severity of any type of rhinitis, especially persistent rhinitis, however, our finding supports the facts that outdoor allergens affect rhinitis significantly [13,20]. Many studies have shown that pollen such as *Artemisia vulgaris* and *Ambrosia artemisifolia* is a larger allergen compared with HDMs and is mainly deposited in the upper airway where it induces local inflammatory or pathological changes, whereas enzymatic activity of pyroglyphid mites seems to be important in the pathogenicity of lower airway and systemic inflammations [34,35]. We have extended this observation by demonstrating the same associations for Chinese weed grass pollens *Artemisia vulgaris* and *Ambrosia artemisifolia* within the group of patients defined as atopic using standard definitions [17]. These findings also indicate that IgE-mediated sensitization is not dichotomous in its relation to the expression, severity and temporal pattern of upper and lower respiratory allergic diseases.

In this study, we also found by both skin test and sIgE measurements that patients with sensitizations to multiple allergens were significantly more likely to have more severe rhinitis and asthma. Our results are in agreement with the study by Simpson et al. [36]. They investigated a group of adults with asthma showing that sensitization to dust mite, cat, dog, and mixed grasses as well as multiple sensitizations were all independently associated with asthma. The data of another study [13] suggested that the development of specific IgE response to multiple indoor allergens is an important factor in the persistence of bronchial obstruction in children with asthma.

In summary, the results of the current study emphasize the importance of sensitization to indoor allergens in asthma severity and to outdoor allergens in severity of rhinitis. Sensitization to more than one allergenic source also significantly increases the possibility of developing moderate-severe rhinitis and asthma.

Acknowledgements

Members of China Alliance of Research on Respiratory Allergic Disease (CARRAD) include Jing Li, Chunqing Zhang, Hongyu Wang, Dehui Chen, Baoqing Sun, Ying Huang, Xiaoping Lin, Deyu Zhao, Guolin Tan, Jinzhun Wu, Changqing Zhao, Jing Zhao, Ling Cao, Guangfa Wang, Changchun Sun, Dongdong Zhu, Xiaoqin Zhou, Nan Su, Jiangtao Lin, Xuefen Wang, Jianying Zhou, Wen Li, Huahao Shen, Yijiang Huang, Shi Chen, Xiaofan Liu, Chuangli Hao, Hong Han, Shaohua Chen, Wei Tang, Huanying Wan, Xiaoyan Dong, Yixiao Bao, Luo Zhang, Dehou Xue, Michael D. Spangfort, Nanshan Zhong. All authors contributed equally to this study. The authors thank Professor Gary Wing-kin Wong, department of paediatrics, The Chinese University of Hong Kong and Birgitte Gjesing, ALK-Abello A/S for help in editing the manuscript; Ms. Mei Jiang, Guangzhou Institute of Respiratory Disease, Guangzhou Medical College, China, for assistance in statistical considerations. This study was supported by ALK-Abello A/S (FC409(10441)-ALK).

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Authors' contributions

JL mainly designed the study, performed the survey, collected the data, performed the statistical analysis and the drafted the manuscript. YH participated in designing the study, performed the survey, collected the data and drafted the manuscript. XL participated in designing the study, performed the survey, and collected the data. DZ participated in designing the study, performed the survey and collected the data. GT performed the survey, collected the data. JW participated in designing the study, performed the survey and collected the data, HZ performed the survey and collected the data. JZ participated in designing the study, performed the survey and collected the data. MS designed the study, performed the statistical analysis and the drafted the manuscript. NZ mainly designed the study, performed the statistical analysis and the drafted the manuscript. All members of China Alliance of Research on Respiratory Allergic Disease participated in discussion the protocol of the study, perform the survey and collected the data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 28 March 2011 Accepted: 15 July 2011
Published: 15 July 2011

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European Journal of Pharmaceutical Sciences 44 (2011) 392–398

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Inhibition of enterovirus 71 replication by chrysosplenetin and penduletin

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ARTICLE INFO

Article history:
Received 4 July 2011
Received in revised form 25 August 2011
Accepted 28 August 2011
Available online 5 September 2011

Keywords:
Enterovirus
Laggera pterodonta
Flavonol
Antiviral activity

ABSTRACT

In recent years, enterovirus 71 (EV71) infections have caused an increasing epidemic in young children, accompanying with more severe nervous system disease and more deaths. Unfortunately, there is no specific medication for it so far. Here we investigated the anti-EV71 activity of chrysosplenetin and penduletin, two *o*-methylated flavonols isolated from the leaves of *Laggera pterodonta*. These two compounds were found to have strong activity in vitro against EV71 with low cytotoxicity. In the cytopathic effect (CPE) inhibition assays, both plaque reduction assay and virus yield inhibition assay, the compounds showed a similar 50% inhibitory concentration (IC_{50}) value of about 0.20 μM. The selectivity indices (SI) of chrysosplenetin and penduletin were 107.5 and 655.6 in African green monkey kidney (Vero) cells, and 69.5 and 200.5 in human rhabdomyosarcoma (RD) cells, accordingly. The preliminary mechanism analysis indicates that they function not through blocking virus entry or inactivating virus directly but inhibiting viral RNA replication. In the time-of-addition assay, both compounds inhibited progeny virus production and RNA replication by nearly 100% when introduced within 4 h post infection. In addition to EV71, both compounds inhibited several other human enteroviruses with similar efficacy. These findings provide a significant lead for the discovery of anti-EV71 drug.

1. Introduction

Human enteroviruses belong to the *Enterovirus* genus of the Picornaviridae family and comprise a major subgroup of small RNA(+) viruses (Palacios and Oberste, 2005), which includes polioviruses, coxsackieviruses, echoviruses, and enteroviruses. Most of these viruses establish infection via the intestinal epithelial tract and lymphoid tissues, and cause paralytic disease, aseptic meningitis, exanthems, myocarditis, pericarditis, and nonspecific febrile illness. Enterovirus 71 (EV71) infections frequently manifest as hand, foot, and mouth disease, as well as encephalitis in infants and young children. EV71 can even cause severe central nervous system disease, complications, and fatalities (Huang et al., 1999; Lee et al., 2009; Solomon et al., 2010). Since it was first reported in 1974 (Schmidt et al., 1974), EV71 has been implicated in several outbreaks worldwide, especially in the Asia–Pacific region (Ooi et al., 2010; Weng et al., 2010). According to the latest reports from the Chinese Ministry of Health, there were over 1 million reported cases of EV71 infection in China in 2010, including 15,000 severe cases and more than 600 fatalities (Ministry of Health of the People's Republic of China, 2010).

At present, there is no specific treatment available for EV71 (Ooi et al., 2010). In the face of frequent outbreaks and the fatal consequences of EV71 infection, antiviral drugs for EV71 are urgently needed. Previous studies indicate that ribavirin and Type I interferon reduces mortality in EV71-infected mice (Liu et al., 2005; Li et al., 2008). Pleconaril, a Sterling-Winthrop (WIN) compound targeting viral protein 1 (VP1), is a successful clinical candidate for most enteroviruses, but not EV71 (Pevear et al., 1999; Shia et al., 2002; Florea et al., 2003; De Palma et al., 2008). On the other hand, pyridyl imidazolidinones derived from WIN compound templates show promising anti-EV71 activity in tissue cultures (Shia et al., 2002; Chen et al., 2009).

Laggera pterodonta has been used as traditional medicine in China for several centuries for its anti-inflammatory and antibacterial properties (Jiangsu New Medical College, 1977). *L. pterodonta* belongs to Compositae and is widely distributed in Southwestern China, especially in Yunnan and Sichuan Provinces. A number of chemical constituents have been isolated from this plant, mainly flavones and triterpenes (Liu et al., 2010). Chrysosplenetin and penduletin are two known flavonols that have been isolated from the leaves of *L. pterodonta* (Li and Ding, 1994). Besides penduletin has been reported to have anti-poliovirus activity and anti-inflam

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doi:10.1016/j.ejps.2011.08.030

Appl Microbiol Biotechnol
DOI 10.1007/s00253-011-3496-8

METHODS AND PROTOCOLS

LAMP-based method for a rapid identification of *Legionella* spp. and *Legionella pneumophila*

Xi Lu · Zi-Yao Mo · Hong-Bo Zhao · He Yan · Lei Shi

Received: 4 May 2011 / Revised: 8 July 2011 / Accepted: 18 July 2011

Abstract *Legionella pneumophila* is accounted for more than 80% of *Legionella* infection. However it is difficult to discriminate between the *L. pneumophila* and non-*L. pneumophila* species rapidly. In order to detect the *Legionella* spp. and distinguish *L. pneumophila* from *Legionella* spp., a real-time loop-mediated isothermal amplification (LAMP) platform that targets a specific sequence of the 16S rRNA gene was developed. LS-LAMP amplifies the fragment of the 16S rRNA gene to detect all species of *Legionella* genus. A specific sequence appears at the 16S rRNA gene of *L. pneumophila*, while non-*L. pneumophila* strains have a variable sequence in this site, which can be recognized by the primer of LP-LAMP. In the present study, 61 reference strains were used for the method verification. We found that the specificity was 100% for both LS-LAMP and LP-LAMP, and the sensitivity of LAMP assay for *L. pneumophila* detection was between 52 and 5.2 copies per reaction. In the environmental water samples detection, a total of 107 water samples were identified by the method. The culture and serological test were used as reference methods. The specificity of LS-LAMP and LP-LAMP for the samples detection were 91.59% (98/107) and 93.33% (56/60), respectively. The sensitivity of LS-LAMP and LP-LAMP were 100% (51/51) and 100% (18/18). The results suggest that real-time LAMP, as a new assay, provides a specific and sensitive method for rapid detection and differentiation of *Legionella* spp. and *L. pneumophila* and should be utilized to test environmental water samples for increased rates of detection.

Keywords Loop-mediated isothermal amplification · *Legionella pneumophila* · Different diagnosis

Introduction

Legionella pneumophila, the agent of Legionnaires' disease and Pontiac fever, is ubiquitous in natural freshwater environments, and it is also present in man-made water systems (Dusserre et al. 2008; Steinert et al. 2007; Albert-Weissenberger et al. 2007). Due to the close relationship between the growth of *L. pneumophila* and human activities (Rowbotham 1980), infection is usually caused by inhalation of aerosols, produced by showers, air conditioning systems, and other aerosol-generating devices (Fields et al. 2002). Governments of various countries have recognized the harmfulness of this type of microorganism. For example, due to the positive result of *L. pneumophila* in the ventilation of public places, it has not been permitted since 2003 in China. Thus, the real-time monitoring of *L. pneumophila* from water systems, particularly in the public places such as hospitals and hotels, is essential for the prevention of *legionellosis* outbreaks (Aurell et al. 2004; Hilbi et al. 2010).

To date, at least 23 *Legionella* species can be recognized as human illness agents. However, more than 80% of *Legionella* infection is attributed to *L. pneumophila* (Yanez et al. 2005). Non-*L. pneumophila* species also have been reported to be infectious, but this happens at very low probability (Herwaldt et al. 1984; Gobin et al.

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Published online: 17 August 2011

Journal of Surgical Oncology 2011;104:162–168

Long-Term Outcome and Cost-Effectiveness of Complete Versus Assisted Video-Assisted Thoracic Surgery for Non-Small Cell Lung Cancer

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Background: To compare the outcomes and costs of two methods of video-assisted thoracoscopic surgery (VATS) major pulmonary resection in patients with clinically resectable non-small cell lung cancer (NSCLC).

Methods: Between January 2000 and December 2007, 1,058 patients with proven stages I–IIIA NSCLC underwent complete VATS (c-VATS) or assisted VATS (a-VATS) major pulmonary resection together with a systematic nodal dissection.

Results: The study cohort consisted of 736 men and 322 women. Mean operative time was shorter for the a-VATS cohort compared with the c-VATS group ($P = 0.038$). Overall survival (OS) at 5 years based on Kaplan–Meier analysis was 55.3% (95%CI, 50.6–60.0%) for those who underwent c-VATS and 47.7% (95%CI, 41.2–54.2%) for those who underwent a-VATS ($P = 0.404$). Gender, final pathology, TNM stage, and pT status were significant predictive factors for OS according to multivariate analysis. The total cost of a-VATS lobectomy was lower than that of c-VATS lobectomy.

Conclusions: c-VATS and a-VATS yield similar results in patients with clinically resectable NSCLC. a-VATS, however, may be less expensive and easier to adopt, making it a particularly attractive option for thoracic surgeons in developing countries.

J. Surg. Oncol. 2011;104:162–168.

KEY WORDS: non-small cell lung cancer; video-assisted thoracoscopic surgery; long-term outcomes; cost-effectiveness

INTRODUCTION

Video-assisted thoracoscopic surgery (VATS) lobectomy is a minimally invasive technique of anatomic pulmonary resection, a procedure which remains the gold standard for the surgical management of non-small cell lung cancer (NSCLC). Meta-analyses [1,2], randomized trials [3–7], case–control series [8,9], and large retrospective series [10,11] have all provided support for the safe and effective use of this minimally invasive technique. However, there are still discrepancies between the method of implementing VATS lobectomy in different centers [12]. Yim et al. [13] conducted a survey of minimally invasive thoracic surgeons in an effort to define their criteria for a VATS lobectomy. The results varied greatly: the numbers of incisions varied from three to five, the utility incision ranged from 4 to 10 cm, and the avoidance of rib spreading was not routine. A number of surgeons perform the procedure using direct visualization through the utility incision, using the thoracoscope merely as a light source, whilst others perform the procedure under total thoracoscopic visualization. Complete VATS (c-VATS) has therefore been described as a purely endoscopic technique with 100% monitor visualization and without rib-spreading minithoracotomy, whereas assisted VATS (a-VATS, also called hybrid VATS) involves performance of the main procedures via rib spreading (rigid or soft spreading) minithoracotomy (5–10 cm long) with both monitor and direct visualization [14,15]. It is not clear if the variability in VATS techniques has contributed to any confusion regarding its efficacy in the management of lung cancers [6,15].

The VATS lobectomy program at Guangzhou Medical College First Affiliated Hospital began in 1994 for selected patients with stage I primary lung cancers. After an initial learning-curve experience with the procedure, we adopted this approach (either c-VATS or a-VATS lobectomy) routinely for the resection of NSCLC in all clinically resectable cases (stages I–IIIA) [16,17], as well as for solitary fibrous tumors of the pleura [18]. In contrast to c-VATS lobectomy, hand-suturing techniques, traditional instruments, and conventional approaches for anatomic dissection can be used conveniently during a-VATS, which promotes a more rapid adoption of this minimally invasive approach, potentially reducing operating times, and minimizing the need for expensive disposable endoscopic products. Accurate financial analyses of new medical technologies are particularly important in today's cost-conscious health care environment [19]. The present study therefore compared not only the long-term survival outcomes of c-VATS and a-VATS lobectomy techniques, but also their economic impact on patients with NSCLC.

Grant sponsor: National Natural Science Foundation of China; Grant number: 30471997; Guangdong Province Science and Technology Planning Programme; Grant number: 2008A030201024; Guangdong Province Science and Technology Key Programme; Grant number: 2007-B031515017.Grant sponsor: Guangzhou City Science and Technology Planning Programme; Grant number: 2007Z3-E0261.

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Received 3 October 2010; Accepted 14 February 2011

DOI 10.1002/jso.21908

Published online 8 March 2011 in Wiley Online Library (wileyonlinelibrary.com).

Surg Endosc (2011) 25:2509–2515
DOI 10.1007/s00464-011-1576-6

Long-term outcome of hybrid surgical approach of video-assisted minithoracotomy sleeve lobectomy for non-small-cell lung cancer

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Received: 4 November 2010 / Accepted: 13 December 2010 / Published online: 7 February 2011

Abstract
Background The aim of this study was to evaluate the technical feasibility and safety of a hybrid surgical approach of video-assisted minithoracotomy (hybrid VATS) sleeve lobectomy for non-small-cell lung cancer (NSCLC), using success rate as the primary end point.
Methods Between February 1996 and December 2006, patients with bronchogenic tumors were prospectively registered to undergo hybrid VATS sleeve resection in a single institution. Hybrid VATS involved performing the main procedures via rib spreading and minithoracotomy using a monitor and direct vision. A successful procedure was defined as a patient who had a sleeve lobectomy via hybrid VATS without conversion to thoracotomy and without significant perioperative morbidity or mortality.
Results A total of 148 patients (108 men and 40 women; median age = 58 years) who underwent hybrid VATS sleeve lobectomy for NSCLC were identified in our database. The median duration of the successfully completed procedures was 190 min (range = 145–305 min). The median length of time of chest tube in place was 3 days (range = 1–12 days). Hybrid VATS sleeve lobectomy was performed successfully in 134 of 148 patients for a success rate of 90.5%. The median follow-up period was 65.1 months (range = 34.5–154.8 months). The overall 5-year disease-free survival and overall survival of all patients were 36.7% (95% CI = 27.9–45.5%) and 54.2% (95% CI = 44.8–63.6%), respectively.
Conclusion Hybrid VATS sleeve lobectomy is feasible for selected patients with NSCLC in specialized centers.

Keywords Non-small-cell lung cancer · Video-assisted thoracic surgery · Hybrid · Sleeve lobectomy

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Sleeve lobectomy is the resection of a portion of a main stem bronchus in continuity with the adjacent lobe or bilobe followed by end-to-end bronchial anastomosis. Since its introduction in 1947 at the Brompton Hospital in London by Sir Clement Price Thomas [1], sleeve lobectomy has been increasingly used for patients with centrally located non-small-cell lung cancers (NSCLC) and low-grade neoplasms, regardless of pulmonary function. Sleeve lobectomy has emerged as an established alternative to pneumonectomy in numerous reports [2–5]. A recent meta-analysis demonstrated that sleeve lobectomy provides better long-term survival and quality of life compared to pneumonectomy in patients with NSCLC [2].

Although video-assisted thoracic surgery (VATS) is regarded as a minimally invasive procedure with good long-term survival outcomes [6], many surgeons consider that the technical limitations of a VATS procedure prohibit

Carcinogenesis vol.32 no.5 pp.713–722, 2011
doi:10.1093/carcin/bgr035
Advance Access publication February 24, 2011

Low-level expression of *let-7a* in gastric cancer and its involvement in tumorigenesis by targeting *RAB40C*

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Gastric cancer is the fourth most common cancer and the second leading cause of cancer mortality worldwide but the underlying molecular mechanism is not entirely clear. The objective of this study was to explore the role of *let-7a* microRNA (miRNA) in gastric tumorigenesis and the possible correlation between *RAB40C* and *let-7a* miRNA in gastric cancer. We found that expression of *let-7a* is reduced in human gastric cancer tissues and cell lines and there was a significant correlation between the level of *let-7a* expression and the stage of differentiation. Overexpression of *let-7a* resulted in a decrease in cell proliferation and G_1 arrest, significantly suppressed anchorage-dependent growth *in vitro* and the tumorigenicity of gastric cancer cells in a nude mouse xenograft model. Furthermore, we demonstrated that *RAB40C* is regulated directly by *let-7a* and plays an essential role as a mediator of the biological effects of *let-7a* in gastric tumorigenesis. This study revealed that *let-7a* is significant in suppressing gastric cancer growth *in vivo* and *in vitro* and provided the first evidence that *RAB40C* is negatively regulated by *let-7a* at the posttranscriptional level via binding to the 3′-untranslated region of *RAB40C* messenger RNA in gastric cancer. The results of this study suggest that *let-7a* and *RAB40C* are potentially useful targets for gastric cancer diagnosis and therapy.

Introduction

Gastric cancer is the fourth most common cancer and the second leading cause of cancer mortality worldwide despite a decreasing incidence in recent decades (1). It remains an important public health burden worldwide, especially in developing countries. In China, gastric cancer has the highest mortality among all cancers and the overall mortality rate has increased steadily in the past 20 years (2). However, the molecular mechanisms involved in gastric cancer are diverse, complex and not fully understood.

New opportunities in the study of cancer molecular mechanisms have been provided by the discovery of microRNAs (miRNAs), a class of short non-coding endogenous RNAs that function as negative regulators of gene expression (3). As the major endogenous triggers for posttranscriptional silencing, miRNAs can negatively regulate the expression of a protein-coding gene by binding with the 3′-untranslated regions (3′-UTRs) of their messenger RNA (mRNA) targets and then repressing expression of the target gene through mRNA degradation or translational inhibition (4,5). miRNAs are predicted to target more than one-third of human genes and each miRNA can control hundreds of target genes (6). Moreover, miRNAs have been demonstrated to be evolutionarily conserved and to perform regulatory functions in numerous biological processes, including developmental timing, cell proliferation, apoptosis, metabolism, cell differentiation and morphogenesis (7–9).

Recently acquired evidence demonstrates that miRNAs can be regulators in carcinogenesis. Calin *et al.* (10) showed that >50% of the known mature human miRNA genes are located in cancer-associated genomic regions or in fragile sites, suggesting that miRNAs might have an important role in the pathogenesis of human cancers. Moreover, different cancer types have distinct miRNA expression profiles, and an increasing number of miRNAs have been suggested to have important roles in tumor progression or in tumor suppression (11–13). Increased expressions of some miRNAs, such as *miR-21* and *miR-27a*, have been found to play crucial roles in gastric tumors (14,15). In addition, the miR-106b-25 cluster, which is upregulated in human gastric tumors, is involved in the posttranscriptional regulation of transcription factor E2F1 (16) and miR-15b and miR-16 modulate multi-drug resistance by targeting B-cell lymphoma/leukmia-2 (BCL2) in human gastric cancer cells (17). In contrast, *miR-9*, *miR-141*, *miR-143*, *miR-145*, *miR-433* and *miR-451* are downregulated in gastric cancer and these miRNAs act as anti-oncogenic miRNAs with a significant growth inhibitory effect on gastric cancer (18–21).

Among all human cancer-related miRNAs, the *let-7* family has attracted the most interest because its family members have been noted to express aberrantly in human cancers (22,23). The family was discovered initially in *Caenorhabditis elegans* and is currently one of the most important members of the miRNA family. The *let-7* family consists of 11 very closely related genes and many human *let-7* genes map to regions that are altered or deleted in human tumors, indicating that these genes might function as tumor suppressors (22). Moreover, when overexpressed in colon cancer cells, *let-7* miRNA leads to growth proliferation associated with a reduced level of *RAS* protein (24). *let-7a* is downregulated in Burkitt's lymphoma and it has been shown to be an anticancer miRNA that repressed *C-MYC* expression at the translational level (25). Recently, the implication of *let-7* in carcinogenesis has been extended to the repression of high-mobility group A2, thus preventing oncogenic transformation in many tumors (26,27). These findings suggest that *let-7* miRNAs participate actively in tumorigenic processes and the targets involved in the regulation of *let-7* miRNAs have been associated with various tumorigenic processes in addition to the miRNAs themselves. However, the data for the relationship between gastric carcinogenesis and the expression of *let-7a* miRNA are very limited. Evidence collected to date shows *let-7a* was linked to the modulation of different target genes, the most well-known being the *RAS* family. The *RAS* proteins function as the critical molecular switch for various signaling pathways controlling the diverse biological processes. *RAB40C* is a member of the *RAS* family, which plays important roles in tumorigenesis. With the help of a bioinformatic analysis, we found *RAB40C* contained the *let-7a* binding site and was evolutionarily conserved across 10 species. To our knowledge, there is no report of work investigating the role of *let-7a* or a possible correlation between *RAB40C* and *let-7a* miRNA in gastric cancers.

Abbreviations: CCK-8, cell counting kit-8; DMEM, Dulbecco's modified Eagle's medium; inhibitor NC, inhibitor non-specific control miRNA; mimic NC, mimic non-specific control miRNA; mRNA, messenger RNA; miRNA, microRNA; PCR, polymerase chain reaction; RT, reverse transcription; siRNA, small interfering RNA; 3′-UTR, 3′-untranslated region.

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Gene 479 (2011) 47–56

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Mechanistic insights into the roles of three linked single-stranded template binding residues of MMLV reverse transcriptase in misincorporation and mispair extension fidelity of DNA synthesis

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ARTICLE INFO

Article history:
Accepted 13 February 2011
Available online 19 February 2011

Received by A.J. van Wijnen

Keywords:
Fidelity
HIV-1
Kinetics
Moloney murine leukemia virus
Reverse transcriptase
Structure–function

ABSTRACT

To obtain some insights into the structure–function relationship of Moloney murine leukemia virus (MMLV) reverse transcriptase (RT), we modeled the catalytic state ternary complexes of this protein using the corresponding RT from human immunodeficiency virus type 1 (HIV-1) and available structures of MMLV RT. We observed that three MMLV RT single-stranded template binding residues, Y64, D114, and R116, act as a linked set through mutual interactions, including hydrogen bonding and ion-pairing. The analogous residues of HIV-1 RT have a somewhat different environment and they lack this linked phenomenon. To understand the functional implication of this linked set of MMLV RT, we performed site-directed mutagenesis at these three positions. Then the mutant enzymes were examined for their biochemical properties and nucleotide selectivity. Mutagenesis of these residues (Y64A, D114A, and R116A) resulted in enzymes with slight to modest changes in polymerase activities. The processivity of DNA synthesis correlated positively with the binding affinity of the MMLV RT variants. Lower fidelity in mutants was indicated by measurements of misincorporation and mispair extension fidelity of wild type (WT) and mutant RTs, in contrast to earlier works that indicate that mutations at the analogous positions in HIV-1 RT result in relatively higher fidelity. These data together with structural analysis suggest that this structural set may therefore be a key factor responsible for the different fidelity of these two RTs.

1. Introduction

Reverse transcriptase (RT) plays a pivotal role as a replicative polymerase for retrovirus survival. The specific RTs of Moloney murine leukemia virus (MMLV) and human immunodeficiency virus type 1 (HIV-1) are the most extensively characterized members of the RT family, both biochemically and structurally. MMLV and HIV-1 RTs share many similarities in their structural and mechanistic characteristics (Goff, 1990; Temin, 1993); however, several key differences also exist. Firstly, MMLV RT is a monomeric 75-kDa enzyme (Moelling, 1974); while HIV-1 RT contains two subunits, p66 and p51 (Le Grice, 1993). Secondly, definitive differences in the catalytic mechanism of the two enzymes exist as judged by the different inactivation patterns obtained with the mutations at conserved sites and the significantly dissimilar kinetic values calculated for the DNA polymerizing functions of both enzymes (Halvas et al., 2000a; Harris et al., 1998; Skasko et al., 2005). Thirdly, the most intriguing difference is that HIV-1 RT represents the lowest fidelity RT of all the retroviruses, while MMLV RT represents the highest fidelity (Bakhanashvili and Hizi, 1992a,b; Roberts et al., 1988; Skasko et al., 2005). We hope that information and insights gained from MMLV RT by structure–function studies as well as deeper comparison of conserved and unique features between these two RTs would help in more profoundly elucidating fundamental mechanisms, especially the fidelity mechanism of RTs.

Biochemical data generated using site-directed mutagenesis and structural studies on HIV-1 RT have shown that the polymerase interactions important to fidelity occur primarily within the nascent base pair binding pocket, especially with the incoming nucleotide, with the minor groove edges of the templating nucleotide and the primer–terminal base pair, and with the single-stranded template strand nucleotides (Kunkel, 2004). The fidelity studies of MMLV RT have been mainly focused on the interactions with the incoming nucleotide, such as the YVDD motif, F155, and Q190 (Gao et al., 1997; Halvas et al., 2000a,b; Kaushik et al., 2000; Pelemans et al., 2001;

Abbreviations: MMLV, Moloney murine leukemia virus; RT, reverse transcriptase; HIV-1, human immunodeficiency virus type 1; WT, wide type.

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0378-1119/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2011.02.007

Published online 21 April 2011 *Nucleic Acids Research, 2011, Vol. 39, No. 15* **6669–6678**
doi:10.1093/nar/gkr232

MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells

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Received October 19, 2010; Revised March 22, 2011; Accepted April 1, 2011

ABSTRACT

microRNAs play an important roles in cell growth, differentiation, proliferation and apoptosis. They can function either as tumor suppressors or oncogenes. We found that the overexpression of miR-192 inhibited cell proliferation in A549, H460 and 95D cells, and inhibited tumorigenesis in a nude mouse model. Both caspase-7 and the PARP protein were activated by the overexpression of miR-192, thus suggesting that miR-192 induces cell apoptosis through the caspase pathway. Further studies showed that retinoblastoma 1 (RB1) is a direct target of miR-192. Over-expression of miR-192 decreased RB1 mRNA and protein levels and repressed RB1-3′-UTR reporter activity. Knockdown of RB1 using siRNA resulted in a similar cell morphology as that observed for overexpression of miR-192. Additionally, RB1-siRNA treatment inhibited cell proliferation and induced cell apoptosis in lung cancer cells. Analysis of miRNA expression in clinical samples showed that miR-192 is significantly downregulated in lung cancer tissues compared to adjacent non-cancerous lung tissues. In conclusion, our results demonstrate that miR-192 is a tumor suppressor that can target the RB1 gene to inhibit cell proliferation and induce cell apoptosis in lung cancer cells. Furthermore, miR-192 was expressed at low levels in lung cancer samples, indicating that it might be a promising therapeutic target for lung cancer treatment.

INTRODUCTION

microRNAs (miRNAs) are single-stranded non-coding small RNAs of ~22 nt that can regulate gene expression in animals, plants and viruses (1). miRNAs are first transcribed by RNA polymerase II as primary miRNAs (pri-miRNAs) that are several thousand nucleotides long (2,3). Pri-miRNAs are processed by the microprocessor complex, which is composed of the RNase III type enzyme, Drosha and the double-stranded RNA binding protein, DiGeorge syndrome critical region gene 8 (DGCR8), to generate ~70 nt precursor miRNAs (pre-miRNAs) with hairpin-shaped structures (4,5). These pre-miRNAs are exported to the cytoplasm by exportin-5 (Exp-5) and the cofactor Ran-GTP (6). In the cytoplasm, pre-miRNAs are processed into 22 nt mature miRNA duplexes by the RNase III Dicer enzyme (7). Mature miRNAs are incorporated into 'miRNA-containing RNA-induced silencing complex (miRISC), which induce either cleavage or translational repression of targeted mRNAs (1,8). The miRNA database (miRBase16.0) contains 1048 records, and the number of known miRNAs is still growing (http://microrna.sanger.ac.uk) (9).

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

TOXICOLOGICAL SCIENCES **119(1)**, 50–60 (2011)
doi:10.1093/toxsci/kfq306
Advance Access publication October 1, 2010

miR-106a–mediated Malignant Transformation of Cells Induced by Anti-benzo[*a*]pyrene-*trans*-7,8-diol-9,10-epoxide

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Received April 20, 2010; accepted September 28, 2010

microRNAs (miRNAs) are an abundant class of small noncoding RNAs that function primarily as oncogenes and tumor suppressors by mediating translational repression or mRNA degradation via binding target genes. In this study, malignant human bronchial epithelial cells transformed by anti-benzo[*a*]pyrene-*trans*-7,8-diol-9,10-epoxide were used to help characterize the possible mechanisms of miRNA function in chemical carcinogenesis. The expression level of miR-106a was measured by the real-time, reverse transcriptase polymerase chain reaction. We used the miR-106a inhibitor and the miR-106a mimic to downregulate or upregulate miR-106a activity in malignantly transformed cells to determine the effects of miR-106a on the biological properties of the cell. We observed overrepresentation of miR-106a in transformed cells compared with control cells. Silencing miR-106a by transfection with the miR-106a inhibitor suppressed cell proliferation, induced cell cycle arrest and apoptosis, and inhibited anchorage-independent growth and tumor growth in nude mice. Increasing miR-106a in malignantly transformed cells by transfection with the miR-106a mimic gave the opposite results. Moreover, untransformed cells showed a reduction of cell cycle arrest and apoptosis rate followed by transfection with the miR-106a mimic. Bioinformatic analysis showed that tumor suppressor RB1 is one of predictive targets of miR-106a. We confirmed this target by Western blot and dual luciferase assay. Our findings suggest that miR-106a might function as an oncogene in transformation induced by a chemical carcinogen. Thus, knock down of miR-106a in malignant cells is a potential therapeutic strategy.

Key Words: **anti-BPDE; human bronchial epithelial cell; malignant transformation; microRNA; miR-106a.**

Lung cancer is one of the most common forms of cancer in humans and is the leading cause of cancer-related death worldwide. Most cases of lung cancer are induced by environmental carcinogens, particularly tobacco smoke (Parkin, 2001). The polycyclic aromatic hydrocarbon benzo[*a*]pyrene (B[*a*]P), which is a toxic element in the environment in general and in tobacco smoke in particular, has atherogenic and carcinogenic properties (Duarte and Paschoal, 2006; Pfeifer *et al.*, 2002). B[*a*]P is activated by microsomal enzymes to yield anti-benzo[*a*]pyrene-*trans*-7,8-diol-9,10-epoxide (anti-BPDE), which binds covalently to nuclear DNA forming BPDE-DNA adducts that can initiate carcinogenesis (Kelley *et al.*, 2005; Xie *et al.*, 2003). Cellular studies and animal studies have concluded that there is evidence that an increased risk of tumorigenesis is associated with BPDE adduct formation (de Vries *et al.*, 1997; Sharma *et al.*, 2008; Vayssier-Taussat *et al.*, 2001; Wu *et al.*, 2009). However, the mechanism involved in anti-BPDE–induced tumorigenesis is not fully understood.

Like other cancers, the development of lung cancer is a multistep process including the accumulation of genetic and epigenetic changes (Damps *et al.*, 2001; Minna *et al.*, 2002). microRNAs (miRNAs) are a class of naturally occurring, small noncoding RNAs that control gene expression by targeting mRNAs for translational repression or cleavage (Du and Zamore, 2005; Pillai, 2005). As a new layer of gene regulation, miRNAs have diverse functions, including the regulation of cellular differentiation, proliferation, and apoptosis (Croce and Calin, 2005). Aberrant miRNA expression has been reported frequently in various tumors, including breast cancer (Shimono *et al.*, 2009), leukemia (Zanette *et al.*, 2007), lung cancer (Cho, 2009; Keller *et al.*, 2009), and colon cancer (Akao *et al.*, 2007), indicating that there is a correlation between miRNAs and human malignancy. A malignant transformation model of the human bronchial epithelial cell line 16HBE induced by anti-BPDE has been established in our laboratory. The immortalized 16HBE cell line retains the specific morphology and function of normal human bronchial epithelial cells and provides a suitable resource for studying the molecular pathogenesis of lung cancer. In earlier studies in our laboratory, 55 significantly differentially expressed miRNAs were identified by microarray in transformed 16HBE cells (Shen *et al.*, 2009). We speculated that some of these

Clinical Investigations

Respiration

Respiration 2011;81:294–301
DOI: 10.1159/000317136

Received: December 21, 2009
Accepted after revision: April 5, 2010
Published online: June 26, 2010

Neural Respiratory Drive in Patients with COPD during Exercise Tests

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Key Words
Chronic obstructive pulmonary disease · Diaphragm · Neural respiratory drive · Respiratory muscle function

Abstract
Background: It is unknown whether neural drive is comparable in constant rate and incremental exercise tests. Few data have previously been available to address this question because of the lack of reliable methods to assess neural respiratory drive in patients with chronic obstructive pulmonary disease (COPD). ***Objectives:*** The aims of this study are to determine whether neural respiratory drive during constant rate exercise differs from that during incremental exercise and to determine whether neural respiratory drive was maximal at the end of exhaustive exercise tests. ***Methods:*** We studied sixteen patients with moderate-severe COPD (mean ± SD FEV_1 29 ± 10%). Both diaphragmatic electromyogram (EMG) and transdiaphragmatic pressure were recorded with a combined multipair electrode balloon catheter during incremental and constant (80% of maximal oxygen consumption derived from a prior incremental exercise test) treadmill exercise. Minute ventilation and oxygen uptake were also measured. ***Results:*** Root mean square (RMS) of the diaphragmatic EMG increased gradually without a plateau during incremental exercise, whereas the RMS increased initially and reached a plateau during constant work rate exercise. The RMS of the diaphragmatic EMG at the end of exercise was similar for both incremental and constant work rate exercise (176 ± 42 μV vs. 184 ± 39 μV); these values were 70 and 73% of maximal values recorded over the study. ***Conclusions:*** The pattern of increase in neural respiratory drive during incremental exercise is different to that observed during constant work rate exercise, but both exercise protocols are terminated when the patients achieve a similar but submaximal drive.

Introduction

In patients with chronic obstructive pulmonary disease (COPD), it has previously been shown that transdiaphragmatic pressure (Pdi) increases initially and reaches a plateau during constant work rate cycle ergometry or treadmill exercise, and that diaphragm fatigue does not develop [1–3]. It is, however, difficult to extrapolate the findings of these studies to levels of neural respiratory drive during exercise in COPD because of the lack of reliable methods to measure neural respiratory drive in this patient population. Occlusion pressure ($P_{0.1}$), tidal volume and Pdi [3, 4] have limitations as measures of neural respiratory drive in COPD because disordered ventilatory mechanics, the development of intrinsic positive end

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0025–7931/11/0814–0294$38.00/0

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JOC The Journal of Organic Chemistry

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Organocatalytic Asymmetric Domino Aza-Michael−Mannich Reaction: Synthesis of Tetrahydroimidazopyrimidine Derivatives

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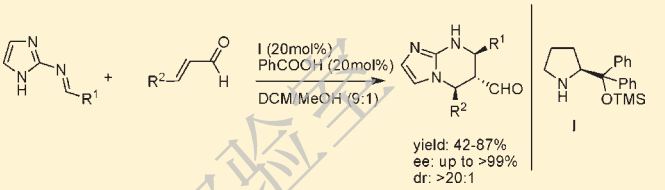
Supporting Information

ABSTRACT: Highly substituted tetrahydroimidazopyrimidine derivatives with three chiral centers have been synthesized for the first time using an organocatalytic asymmetric domino aza-Michael−Mannich reaction of α,β-unsaturated aldehydes and *N*-arylidene-1*H*-imidazol-2-amines. This efficient approach furnishes the products in good yields (42−87%) with excellent stereoselectivities (>20:1 dr, up to >99% ee).

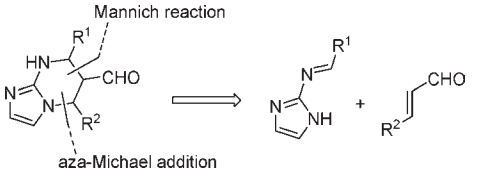


The tetrahydroimidazopyrimidine ring system is found in many naturally occurring products that have attracted attention due to the broad scope of their biological activities.[1,2] Different classes of tetrahydroimidazopyrimidine compounds have shown antidepressant[2a,b] and antihypertonia[2c] activities that have been put to pharmaceutical uses. However, syntheses of tetrahydroimidazopyrimidine derivatives are not very well documented in the literature.[2b,3] The traditional methods for their synthesis often require many synthetic manipulations and purifications, which result in low overall yields. Thus the development of novel, concise methodologies that allow the rapid construction of these tetrahydroimidazopyrimidine skeletons, preferably in a single operation, is highly desired.

Organocatalytic domino reactions[4−6] allow the sequential formation of several new bonds and chiral centers in just one operation. They have been proven to be powerful tools for the efficient and stereoselective synthesis of complex molecules[7] that are difficult to access by traditional methods. The aza-Michael addition[8] participated domino reaction provides a simple and direct way for the synthesis of nitrogen-containing heterocycles. For example, the asymmetric syntheses of 1,2-dihydroquinolines,[9] pyrrolidines,[10] tetrahydro-1,2-oxazines,[11] and isoindolines[12] have been realized. Herein, we report the first asymmetric synthesis of enantioenriched tetrahydroimidazopyrimidine derivatives through an organocatalytic domino strategy using α,β-unsaturated aldehydes and *N*-arylidene-1*H*-imidazol-2-amines as starting materials (Scheme 1).

The reactions of 2-carbonyl-substituted indoles[13] and pyrroles[14] with enals have been realized for the syntheses of pyrrolidine-fused heterocycles. However, the asymmetric synthesis of six-membered ring-fused heterocycles, such as biologically interesting tetrahydroimidazopyrimidines, through aza-Michael reaction of nitrogen heterocycles has not been reported. We would like to focus our research on this challenging task. Unlike tetrazole, triazole, and other nitrogen heterocycles,[15] the N−H group of imidazole is not acidic enough to participate in N-alkyaltion reactions. The introduction of electron-withdrawing groups, such as carbonyl or cyano, can reduce the pK_a value of this N−H group, making it possible for N-alkylation reactions[13,14,15b] to occur. The iminic group is a weak electron-withdrawing group and enables many further transformations. We envisaged that the *N*-arylidene-1*H*-imidazol-2-amines (**1**) and the α,β-unsaturated aldehydes (**2**) might be suitable substrates for the domino aza-Michael−Mannich reaction and that they would generate the highly substituted tetrahydroimidazopyrimidine derivatives (**3**). To test our hypothesis, the readily available L-proline-derived secondary amines (**I**−**IV**), which are capable of both iminium[16] and enamine[17] catalysis, were explored as catalysts for this domino reaction.

Scheme 1. Domino Aza-Michael−Mannich Reaction of α,β-Unsaturated Aldehyde and *N*-Arylidene-1*H*-imidazol-2-amine



The reaction of *N*-benzylidene-1*H*-imidazol-2-amine (**1a**) and cinnamaldehyde (**2a**) was selected as a model reaction. We first studied catalysis of the domino reaction with diphenyl prolinol silyl ether (**I**) in dichloromethane. The reaction proceeded with high stereoselectivity (97% ee, >20:1 dr) but in low yield (30%, Table 1, entry 1). There was no significant improvement in yield when using a variety of different solvents, most

Received: June 22, 2011
Published: August 10, 2011

Official Journal of the Asian Pacific Society of Respirology
Respirology

INVITED COMMENTARY:
RESPIRATORY HEALTH ISSUES IN THE ASIA-PACIFIC REGION

Outdoor air pollution and respiratory health in Asia

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ABSTRACT

With the rapid economic development occurring in the last decade in many countries of Asia, the level of air pollution has increased from both industrial and motor vehicle emissions. Compared with Europe and North America, the potential health effects of this increasing air pollution in Asia remain largely unmeasured. Recent data published by the Health Effects Institute from some major cities in India and China reveal that a 10 μg/m^3 increase in PM_{10} was associated with an increase in mortality of 0.6% in daily all-natural cause mortality, with higher risks being found at extremes of high temperatures and in the lowest economically advantaged population. Other Asian studies have confirmed the link between hospital admissions for the worsening of COPD and the increase in asthma prevalence to levels of outdoor air pollutants. Although potential health effects appear to be similar to already-published Western data, it is important that further studies be carried out in Asia that will inform the public and the authorities of the necessity to curb levels of outdoor air pollutants to acceptable levels.

Key words: cigarette smoking, mortality, outdoor air pollution, respiratory disease, time-series study.

INCREASING AIR POLLUTION IN ASIA

The economic development of Asia is accelerating, particularly in the most populous countries of China and India. Accompanying this increased pace of industrialization and prosperity is severe urban air pollution, which is making many Asian cities among the most polluted in the world.[1] While domestic coal fires, power plants and heavy industry have largely disappeared from many parts of Europe to be replaced by the use of cleaner fuels and advanced emission-control technologies, the situation in developing Asia with regard to these sources of pollution has been worsening.

Along with emissions from continuing practice of burning low-quality (sulphurous) coal both domestically and industrially, photochemical smog resulting from car pollutants consisting of hydrocarbons and nitrogen dioxides (NO_2) interacting in the presence of sunlight is now common in many Asian cities. This is not limited to urban areas as the components of this smog such as ozone and respirable particles $PM_{2.5}$ can travel long distances away from their sources. The overlap of photochemical and sulphurous smog in Asian countries may be important because both the uses of coal and cars are occurring at the same time. Total energy consumption has increased in most countries of Asia, and more so in China, particularly since 2001. In Asia, the amount of coal use has nearly doubled within the last 15 years until 2005, with a 50% increase in consumptions of oil, natural gas and other fuels. The number of vehicles in the whole of Asia is increasing, and in China alone this was in excess of 5 million in 2005. It is projected that there will be nearly three times more vehicles in 2015 compared with 2005, a staggering increase of 250 million vehicles in all.

Air pollution is one of the major factors that affects the health of Asians,[2] contributing to over half a million deaths and 3.1 million lost years of healthy life in Asian developing countries in 2002,[3] representing two-thirds of the global burden of deaths attributed to air pollution. Indoor air pollution contributed to an additional 1.1 million deaths in Asia. Therefore, both outdoor and indoor pollution are important factors

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Received 7 July 2011; invited to revise 27 July 2011; revised: 2 August 2011; accepted 2 August 2011 (Associate Editor: David Hui).

Respirology (2011) **16**, 1023–1026
doi: 10.1111/j.1440-1843.2011.02034.x

ORGANIC LETTERS

2011
Vol. 13, No. 17
4604–4607

Palladium-Catalyzed Intramolecular C(sp^2)–H Amidination by Isonitrile Insertion Provides Direct Access to 4-Aminoquinazolines from *N*-Arylamidines

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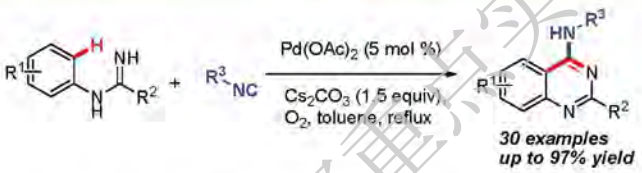
ABSTRACT



An efficient method for the synthesis of 4-amino-2-aryl(alkyl)quinazolines from readily available *N*-arylamidines and isonitriles via palladium-catalyzed intramolecular aryl C–H amidination by isonitrile insertion has been developed.

Transition-metal-catalyzed functionalization of C–H bonds, surrogates of preinstalled C–(pseudo)halogen bonds, serves as an attractive atom-economical and environmentally benign strategy for C–C and C–heteroatom bond formation.[1] Due to the high bond dissociation energies and ubiquity of C–H bonds in organic molecules, the presence of a nearby chelating group is usually required in order to direct positioning of a metal catalyst so that specific C–H bond activation occurs. Consequently, extra steps are required for introduction and removal of directing groups, which offset the merit of C–H functionalization and limit its synthetic applications. However, intramolecular heterofunctionalization of C–H bonds is an ideal, truly atom-economical approach to the construction of heterocyclic architectures, since heteroatoms act as both directing groups and intramolecular nucleophiles

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10.1021/ol201807n
Published on Web 08/05/2011

Phosphoproteome profile of human lung cancer cell line A549†

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Received 17th June 2010, Accepted 22nd September 2010
DOI: 10.1039/c0mb00055h

As an *in vitro* model for type II human lung cancer, A549 cells resist cytotoxicity *via* phosphorylation of proteins as demonstrated by many studies. However, to date, no large-scale phosphoproteome investigation has been conducted on A549. Here, we performed a systematical analysis of the phosphoproteome of A549 by using mass spectrometry (MS)-based strategies. This investigation led to the identification of 337 phosphorylation sites on 181 phosphoproteins. Among them, 67 phosphoproteins and 230 phosphorylation sites identified appeared to be novel with no previous characterization in lung cancer. Based on their known functions as reported in the literature, these phosphoproteins were functionally organized into highly interconnected networks. Western blotting and immunohistochemistry analyses were performed to validate the expression of a bottleneck phosphoprotein YAP1 in cancer cell lines and tissues. This dataset provides a valuable resource for further studies on phosphorylation and lung carcinogenesis.

Introduction

As the most common type of human malignancy, lung cancer is the leading cause of cancer deaths in both women and men throughout the world. One of the major reasons for this are the lack of biomarkers for diagnosing lung cancer at an early stage. Previous investigations have shown that many proteins contribute to the progression of lung cancer, such as phospho-Ser/Thr-Pro specific prolyl-isomerase Pin1,[1] EGFR and PI3K,[2] LKB1,[3] phospho-eIF4E,[4] epithelial and endothelial tyrosine kinase (Etk)[5] and Akt signaling pathways,[2,4] suggesting that the alteration of phosphorylation plays a critical role in tumor development.

Phosphoproteins are involved in diverse signaling pathways in living cells. Reversible phosphorylation of serine, threonine, and tyrosine regulates a series of biological processes including cell growth, differentiation, proliferation, apoptosis, and even intercellular communication.[6] It is well known that phosphorylation is the key process in tumor progression in many cancers including lung cancer. The characterization of phosphorylation status is key to the understanding of cancer development.

Recent developments in MS technology and advances in methods for low-abundance phosphoprotein or phosphopeptide enrichment including immobilized metal affinity chromatography (IMAC),[7] strong cation exchange chromatography (SCX),[8] or the two in combination[9] have enabled large-scale identification of phosphorylation sites. In particular, titanium dioxide (TiO_2) chromatography has been adopted as an efficient method for enriching phosphopeptides from complex samples.[10] TiO_2 columns have a high selectivity for phosphorylated peptides, where unspecific binding with non-phosphorylated peptides can be reduced by including 2,5-dihydroxybenzoic acid (DHB), phthalic acid or glycolic acid with a high concentration of trifluoroacetic acid (TFA) in the loading buffer.[11] In addition, TiO_2 chromatography of phosphorylated peptides is tolerant toward most buffers and salts used in biochemistry and cell biology.[12]

Many studies have demonstrated the power of MS-based proteomics approaches to identify altered proteins as potential lung cancer biomarkers.[13–15] Investigations into phosphorylation events in lung cancer have also been conducted, but were usually restricted to the single protein level, including the phosphorylation of p53,[16] H2AX,[17] and dephosphorylation of STAT3.[18] No large-scale phosphoproteomic analysis has been reported so far.

We have recently described an integrated phosphoproteomic technology coupled with phosphopeptide enrichment to identify phosphoproteomes in primary human multiple myeloma cells.[6] We here extended this approach to study the phosphoproteome of human lung cancer A549 cells by using TiO_2 enrichment in combination with LC-MS/MS analysis. A total of 337 phosphorylation sites on 181 phosphopeptides were identified, including 227 on serine, 87 on threonine and 23 on tyrosine sites. Validation experiments and bioinformatics analysis were performed to elaborate the implication of the phosphorylations in lung cancer. We hope this work can help us further understand the correlation of phosphorylation and lung carcinogenesis.

Results and discussion

Identification of phosphorylation proteins and sites

To selectively enrich low-abundance phosphoproteins and phosphopeptides, experimental conditions were optimized

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† Electronic supplementary information (ESI) available: Table S1–S3 and Supplemental S4. See DOI: 10.1039/c0mb00055h
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PLoS one

Potent Neutralization of Influenza A Virus by a Single-Domain Antibody Blocking M2 Ion Channel Protein

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Abstract

Influenza A virus poses serious health threat to humans. Neutralizing antibodies against the highly conserved M2 ion channel is thought to offer broad protection against influenza A viruses. Here, we screened synthetic Camel single-domain antibody (VHH) libraries against native M2 ion channel protein. One of the isolated VHHs, M2-7A, specifically bound to M2-expressed cell membrane as well as influenza A virion, inhibited replication of both amantadine-sensitive and resistant influenza A viruses *in vitro*, and protected mice from a lethal influenza virus challenge. Moreover, M2-7A showed blocking activity for proton influx through M2 ion channel. These pieces of evidence collectively demonstrate for the first time that a neutralizing antibody against M2 with broad specificity is achievable, and M2-7A may have potential for cross protection against a number of variants and subtypes of influenza A viruses.

Citation: Wei G, Meng W, Guo H, Pan W, Liu J, et al. (2011) Potent Neutralization of Influenza A Virus by a Single-Domain Antibody Blocking M2 Ion Channel Protein. PLoS ONE 6(12): e28309. doi:10.1371/journal.pone.0028309

Editor: Suryaprakash Sambhara, Centers for Disease Control and Prevention, United States of America

Received August 4, 2011; **Accepted** November 6, 2011; **Published** December 2, 2011

Funding: This work was supported in part by the Guangzhou Institute of Biomedicine and Health (GIBH), Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX1-YW-10, KSCX2-EW-J-27), and State Key Laboratory of Respiratory Diseases. No additional external funding was received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

As a serious public health threat, influenza A virus causes seasonal epidemics as well as occasional pandemics. It is estimated that 250,000–500,000 people die from influenza each year throughout the world [1]. The 1918 Spanish influenza pandemic infected close to 5% of the world's population and caused a devastating effect [2]. Recent outbreaks of H1N1 influenza (Swine flu) again raised serious concerns about potential influenza pandemics [3]. Although vaccines and anti-viral drugs are currently available to control influenza, their prophylactic and therapeutic effects remain incomplete. Conventional vaccines mainly target two highly variable determinants; namely, hemagglutinin (HA) and neuraminidase (NA). Due to rapid genetic drift and re-assortment of the viral genome, viral strains evolve continuously and necessitate frequent updates for vaccine production. The time delay from monitoring the emergences of new viral strains to producing effective vaccines at an industrial scale limits our ability to provide immediate protection when a pandemic occurs [4]. In turn, the new vaccines would not be able to provide effective protection for immuno-compromised individuals, young children and the elderly [5]. Besides vaccines, anti-viral drugs such as NA inhibitors zanamivir and oseltamivir as well as matrix-2 protein (M2) inhibitors amantadine and its derivative rimantadine were approved to combat influenza. However, substantial amount of drug-resistant viruses emerged due to frequent use of these drugs. Alarmingly, in humans, birds, and pigs, amantadine-resistant viruses constitute more than 90% of total [6–8]. Thus, there is a pressing need to develop effective prophylactic and therapeutic agents against infection of different variants and subtypes of influenza A viruses.

Influenza M2 is an integral tetrameric transmembrane protein that functions as a proton channel required for uncoating the virus in endosomes upon infection, and hence, a functional M2 is essential for a productive infection to occur [9–12]. Compared to other viral surface proteins such as HA and NA, the 23-amino acid extracellular domain of M2 (M2e) is remarkably conserved in all human influenza A viruses [13]. This distinctive characteristic makes M2e an attractive target for developing a "universal" vaccine. In recent years, several M2e-based vaccines have been demonstrated in animal models to protect against human and avian influenza infections [14–18]. However, inadequate antibody titers are particular challenging due to the low immunogenicity of M2e [19], and multiple injections of high-dose immunogens with an adjuvant are required to achieve high levels of neutralizing antibodies [20]. Passive immunization has been proven to be an effective and safe strategy for the prevention and treatment of viral diseases [21]. Passive transfer of murine anti-M2e antibody 14C2 significantly inhibited influenza A virus replication in mice [22]. Several groups developed M2e monoclonal antibodies (mAbs) and demonstrated their prophylactic and therapeutic activities against influenza [20,23–26]. In general, these antibodies mediate protection by eliminating infected cells through antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), not through neutralizing virions as M2 ion channel blockers [20,27]. Conceivably, blocking M2 ion channel would be an effective antiviral approach since M2 is involved in virus uncoating at an early stage of the viral life cycle. Yet, there is

Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Preliminary X-ray crystallographic analysis of the glycosyltransferase from a marine *Streptomyces* species

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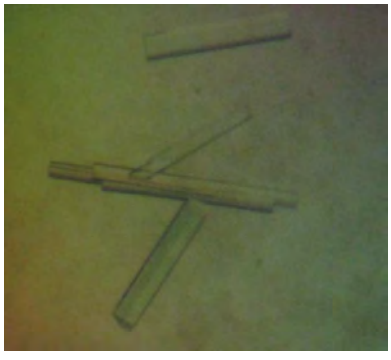
Received 14 October 2010
Accepted 19 November 2010



ElaGT is a glycosyltransferase from a marine *Streptomyces* species that is involved in the biosynthesis of elaiophylin. Here, the molecular cloning, protein expression and purification, preliminary crystallization and crystallographic characterization of ElaGT are reported. The rod-shaped crystals belonged to space group $P2_122$, with unit-cell parameters a = 66.7, b = 131.7, c = 224.6 Å, α = 90, β = 90, γ = 90°. Data were collected to 2.9 Å resolution. A preliminary molecular-replacement solution implied the presence of two ElaGT molecules in the asymmetric unit.

1. Introduction

The polyketide elaiophylin contains an unusual 16-membered ring system comprising two identical 2-deoxy-L-fucoses with *C*2 symmetry (Fig. 1). This natural product has been isolated from many *Streptomyces* species (Fang *et al.*, 2000; Fiedler *et al.*, 1981; Haltli, 2006; Hammann *et al.*, 1990; Haydock *et al.*, 2004) and has also recently been found in the marine *Streptomyces* sp. SCSIO 01934 identified in sediment from the South China Sea (unpublished data). Elaiophylin exhibits broad antibacterial and antifungal activity (Fiedler *et al.*, 1981; Hammann *et al.*, 1990) and, as has often been found for other glycosidic antibiotics (Salas & Mendez, 2007; Thibodeaux *et al.*, 2008; Williams *et al.*, 2008), the deoxy-sugar moieties play an essential role in modulating its bioactivity (Evans & Fitch, 1997).

The attachment of sugar moieties to natural products is generally catalyzed by glycosyltransferases, which have proven to be potential enzymatic tools for enhancing the diversity and activity of natural products (Thibodeaux *et al.*, 2008; Williams *et al.*, 2008; Zhang, Griffith *et al.*, 2006). Elucidation of the elaiophylin-biosynthetic gene cluster identified a single glycosyltransferase ElaGT (Haydock *et al.*, 2004; Haltli, 2006) which is putatively responsible for the sequential attachment of the two deoxy sugars. Iteratively acting glycosyltransferases have been demonstrated to be involved in the biosynthesis of landomycin and avermectin (Luzhetskyy *et al.*, 2005; Zhang, Albermann *et al.*, 2006). However, the catalytic mechanism of this class of glycosyltranferases remains unresolved. Structural elucidation of glycosyltransferases has facilitated understanding of their mechanisms of action and substrate specificities (Roychoudhury & Pohl, 2010) and is also important for the rational engineering of glycosyltransferases for glycodiversification (Williams *et al.*, 2008).

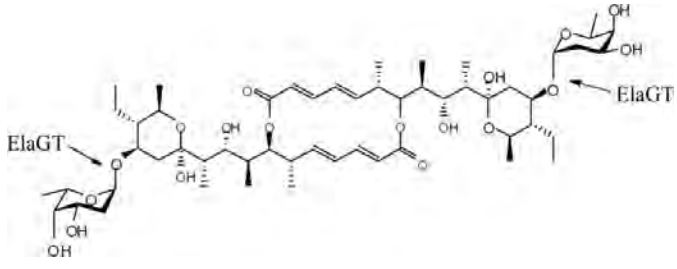


Figure 1
Chemical structure of elaiophylin. Two deoxy sugars are putatively biosynthesized by a single glycosyltransferase ElaGT.

Journal of Surgical Oncology 2011;104:841–846

Prognostic Impact of MMP-2 and MMP-9 Expression in Pathologic Stage IA Non-Small Cell Lung Cancer

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Background: The purpose of the present study was to assess the value of matrix metalloproteinase (MMP)-2 and MMP-9 expression and other potential prognostic factors in predicting the clinical outcome of patients after definitive surgery for pathologic stage IA non-small cell lung cancer (NSCLC).

Methods: One hundred and forty-six consecutive and non-selected patients who underwent definitive surgery for stage IA NSCLC were included in this study. Formalin-fixed paraffin-embedded specimens were stained for MMP-2 and MMP-9, which were statistically evaluated for their prognostic value and other clinicopathological parameters.

Results: Of the 146 patients studied, 102 (69.9%) cases were classified as having high expression for MMP-2. A total of 89 carcinomas (61.0%) had high expression for MMP-9. MMP-9 expression correlated with Eastern Cooperative Oncology Group (ECOG) performance status, pT stage, and differentiation ($P = 0.005$, <0.001, and <0.001, respectively). Vessel invasion, pT stage, and MMP-9 expression maintained their independent prognostic influence on overall survival ($P = 0.037$, <0.001, and <0.001, respectively).

Conclusions: From results of our relatively large database, MMP-9 may be considered as a viable biomarker that can be used in conjunction with other prognostic factors such as vessel invasion and pT stage to predict the prognosis of patients with completely resected pathologic stage IA NSCLC.

J. Surg. Oncol. 2011;104:841–846. © 2011 Wiley Periodicals, Inc.

KEY WORDS: non-small cell lung cancer; MMP-2; MMP-9; vessel invasion

INTRODUCTION

Non-small cell lung cancer (NSCLC) accounts for approximately 75% of all cases of lung cancer, which is one of the most common tumors in the world [1]. Currently, the best prognostic index for operable NSCLC is the tumor-node-metastasis (TNM) staging system. However, this classification scheme can be imprecise as an isolated prognostic factor for an individual patient. Overall 5-year survival rates in patients with resected stage IA disease range from 67 to 89%, indicating a degree of inaccuracy in prognosis due to differences in other factors for patients with same stage [2,3].

Stage IA NSCLC is sub-divided according to poor prognostic factors such as smoking history [4], serum level of carcinoembryonic antigen (CEA) [5], extent of operation [6], tumor size [7,8], and vessel invasion [9]. However, main recent studies have focused on identifying molecular markers of recurrence after surgical treatment which may facilitate the selection of optimal adjuvant chemotherapy treatment for the individual patient [10].

Experimental studies have reported that degradation of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) is a critical process in the progression of malignant tumors, including NSCLC, as degradation of the ECM is essential for tumor angiogenesis, tumor invasion, and development of metastases [11–15]. Amongst many MMPs that have been identified, MMP-2 (Gelatinase-A) and MMP-9 (Gelatinase-B) have been hypothesized to be key enzymes, as they degrade type IV collagen, the main component of ECM [14,15]. After these experimental studies, there has been an intensified interest in many clinical studies on MMP-2 and/or MMP-9 expression in malignant tumors, including NSCLC [16–21]. However, the clinical significance of these findings remains controversial [14]. The aim of the present study was to assess the prognostic value of MMP-2 and MMP-9 expression as well as other potential prognostic factors after definitive surgery of pathologic stage IA NSCLC.

PATIENTS AND METHODS

Between November 2001 and December 2007, 146 consecutive and non-selected patients who underwent definitive surgery for NSCLC at the First Affiliated Hospital of Guangzhou Medical College were found to have pathologic stage IA disease. All patients had histologically confirmed squamous cell carcinoma, adenocarcinoma, bronchioloalveolar carcinoma (BAC), or large cell carcinoma. Small cell lung cancer and cell types of undetermined histology were excluded from the present analysis.

Conflict of Interest: None.

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Received 11 May 2011; Accepted 31 May 2011

DOI 10.1002/jso.22001

Published online 30 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

Original article

Quadriceps strength assessed by magnetic stimulation of femoral nerve in patients with chronic obstructive pulmonary disease

JU Chun-rong and CHEN Rong-chang

Keywords: *chronic obstructive pulmonary disease; magnetic stimulation; quadriceps; twitch tension; maximal volitional contraction*

Background Skeletal muscle dysfunction is one of important systemic manifestations of chronic obstructive pulmonary disease (COPD) and is associated with mortality in patients with COPD, thus quantifying its strength is of great clinical interest and of particular value. Quadriceps maximal volitional contraction (MVC) is often used for the routine measurements of this muscle's strength; while twitch tension (TwQ) evoked by magnetic stimulation of the femoral nerve has been employed for measurement of quadriceps strength non-volitionally. We aimed to investigate the prevalence and severity of skeletal muscle dysfunction in COPD patients by measurement of quadriceps strength with volitional and non-volitional techniques, and to probe into some methodological issues.

Methods We recruited 71 COPD patients and 60 control subjects. Quadriceps strength was measured with both maximality of TwQ and MVC force. The reproducibility for TwQ and MVC was investigated using within-occasion variability from three repeated maneuvers.

Results Maximal TwQ was achieved in 121 participants at a mean of 90% of the stimulator's maximum output. The mean maxmality of TwQ was decrease by about 44%–47% in COPD patients as compared with controls (P <0.05), so was MVC. There was a significant correlation between quadriceps TwQ and MVC, and the mean ratio of TwQ/MVC was 0.29 in controls and 0.33 in patients. The coefficient of variation showed that TwQ yielded lower within-occasion variability than MVC in both groups.

Conclusions Quadriceps strength is commonly and substantially impaired in patients with COPD, in terms of MVC as well as TwQ. The magnetic stimulation of the femoral nerve presents a higher reproducibility and is a better technique for measurement of quadriceps strength for the general population, especially for those who are too unwell to perform a full MVC; while it may not be applied to subjects who are over-weighted.

Chin Med J 2011;124(15):2309-2315

Chronic obstructive pulmonary disease (COPD) is a major medical problem and a leading cause of morbidity and mortality among the adult population worldwide. It is a debilitating disease characterized by inflammation-induced airflow limitation and parenchymal destruction.[1] In addition to pulmonary manifestations, patients with COPD develop systemic problems, including skeletal muscle and other organ-specific dysfunctions.[2] Peripheral skeletal muscle dysfunction has been recognized as one of the most important systemic manifestations of COPD.[3] Recent studies have shown that quadriceps strength can predict mortality not only in patients with COPD[4] but also in patients with heart disease,[5] thus quantifying its strength is of great clinical interest and of particular value. Quadriceps maximal volitional contraction (MVC) is often used for routine measurements of this muscle's strength, while a true MVC relies on subject motivation, cooperation, and learning effects. Over the past few decades, increasing data have suggested that magnetic stimulation of the femoral nerve may have clinical value in the measurement of quadriceps muscle function, which is a new technique for the assessment of quadriceps strength by measurement of twitch tension (TwQ), a test that is independent of subject's motivation.[6] This technique is easy to use to elicit quadriceps TwQ, and the true strength of quadriceps can be exactly reflected by the maximality of TwQ.[7,8] Maximality implies that a further increase in stimulus intensity results in no further increase in TwQ or amplitude of surface electromyography.[9] Therefore, this study was aimed to investigate the prevalence and severity of skeletal muscle dysfunction in COPD by assessment of quadriceps strength with both TwQ and MVC, and to investigate the advantages and limitations of the new technique of magnetic stimulation of the femoral nerve.

METHODS

Subjects selection

Seventy-one patients with stable COPD and 60 age-matched healthy volunteers were recruited for the

DOI: 10.3760/cma.j.issn.0366-6999.2011.15.012

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Sequential Hydration–Condensation–Double Cyclization of Pyridine-Substituted 2-Alkynylanilines: An Efficient Approach to Quinoline-Based Heterocycles

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Received 1 March 2011; revised 26 March 2011

Abstract: An environmentally benign and atom-economical process to construct a unique quinoline-based tetracyclic scaffold, through sequential hydration–condensation–double cyclization reactions, has been described. The reaction starts with readily available pyridine-substituted *o*-alkynylanilines and β-keto esters, promoted by *p*-toluenesulfonic acid in ethanol in one pot. In the absence of β-keto esters, multisubstituted quinolines are formed bimolecularly in reasonable yields.

Key words: alkynes, cyclization, heterocycles, quinoline, tandem reaction

The addition of water to C≡C bonds catalyzed by mercury(II), known as hydration of alkynes for more than a century ago, produces the corresponding carbonyl compounds under mild conditions.[1] Over the last 30 years, much attention has been paid to the development of mercury-free catalysts with improved efficiency and regioselectivity and applying the process to tandem reactions involving carbonyl compounds due to the environmental concern and the convenient accessibility of alkynes.[1] A mixture of two ketones is usually obtained from asymmetric internal alkynes when substitution patterns are similar (Scheme 1, Equation 1). Regioselective hydration of arylalkynes bearing alkyl groups at the other end of the triple bond can be achieved via Brønsted acid catalyzed hydration, affording 2-substituted acetophenones (Scheme 1, Equation 2).[2] For asymmetric diarylalkynes, the selectivity is usually poor unless one of the aromatic ring is electron-rich and the other one is electron-poor or at least electron-neutral. Therefore, the electron-rich *o*-alkynylanilines are ideal precursors for 2-substituted 2′-aminoacetophenones upon hydration in the presence of Brønsted acids (Scheme 1, Equation 3).[3] The resulting 2-substituted 2′-aminoacetophenones can serve as versatile building blocks in the synthesis of N-heterocycles.[3,4]

As part of our continuing interest in diversified quinoline synthesis,[5] we reported a Friedländer type reaction for the synthesis of multisubstituted 4-alkylquinolines from *o*-alkynylanilines and activated ketones in the presence of *p*-toluenesulfonic acid in ethanol under reflux (Scheme 1, Equation 3).[6] When R^2 is a phenyl, the yield is relatively low, which can be explained by the poor regioselectivity during the hydration process. We conceived that higher efficiency could be achieved by introduction of an electron-deficient pyridine at the other end of the triple bond. Furthermore, the pyridine motif could serve as a nucleophile, cyclizing the pyridine nitrogen with the carboxylic ester at C-3 (Scheme 1, equation 4). Thus, additional complexity can be introduced to the quinoline scaffold. In addition, the pyridine moiety is ubiquitously distributed in natural products and synthetic compounds of pharmaceutical interests.[7] And the resulting multicyclic heterocycles containing both pyridine and quinoline moieties are expected to be biologically active.[8] In this proposed reaction, three chemical bonds – C=N, C=C, C–N – will be formed sequentially in one operation with high bond-forming efficiency and atom-economy.

The pyridine-substituted *o*-alkynylanilines **3** with electron-donating and -withdrawing groups on both of the aromatic rings were readily accessible from Sonogashira coupling of *o*-ethynylanilines **1**[5a,b] and substituted 2-bromopyridines **2** in good to excellent yields as outlined in Scheme 2.

The proposed reaction was tested between *o*-[2-(pyridin-2-yl)ethynyl]aniline (**3a**; R^1, R^2 = H) and ethyl acetoacetate (**4a**; R^3 = Me) under the conditions reported previously by us.[6] The desired cyclization did take place by forming an amide bond between the pyridine nitrogen and the carboxylic ester in high yield in the presence of *p*-toluenesulfonic acid (**5a**, Table 1). The unique tetracyclic heterocycle was constructed via sequential formations of three chemical bonds with one molecule of ethanol and water as the only waste products. Substrates with methyl groups at three out of the four possible positions on the pyridine ring were successfully applied (**5b**–**d**, Table 1). 6-Methyl-substituted pyridine failed to cyclize due to the steric hindrance around nitrogen. Surprisingly, the quinoline intermediate before cyclization was not isolated either. Although the nucleophilicity of pyridine nitrogen was reduced by electron-withdrawing groups, such as Cl and CN, corresponding products were obtained in excellent yields (**5e**–**f**, Table 1). And more importantly, these functionalities provided handles for further modification on the scaffold. The fluoro substitution at C-5 also delivered the corresponding product **5g** in reasonable yield. The unprecedented pentacyclic heterocycle containing a

SYNTHESIS 2011, No. 11, pp 1723–1732
Advanced online publication: 15.04.2011
DOI: 10.1055/s-0030-1260001; Art ID: H23611SS

Molecules **2011**, *16*, 3222-3231; doi:10.3390/molecules16043222

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

Structure Identification of Euphorbia Factor L3 and Its Induction of Apoptosis through the Mitochondrial Pathway

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Received: 9 February 2011; in revised form: 4 April 2011 / Accepted: 12 April 2011 / Published: 15 April 2011

Abstract: In this article, we have focused on the structure identification of Euphorbia factor L3 belonging to the lathyrane diterpenoids isolated from Caper Euphorbia Seed. Its anticancer activity *in vitro* against lung cancer A549 cells was also investigated and the IC_{50} values were 34.04 ± 3.99 μM. Furthermore, Euphorbia factor L3 could induce apoptosis in A549 cells via the mitochondrial pathway including loss of mitochondrial potential and release of cytochrome *c*.

Keywords: euphorbia factor L3; caper euphorbia seed; apoptosis; mitochondrial pathway

RESEARCH ARTICLE

Subcellular proteomics revealed the epithelial–mesenchymal transition phenotype in lung cancer

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Received: December 12, 2009
Revised: November 10, 2010
Accepted: November 10, 2010

Subcellular proteomics was used to compare the protein profiles between human lung adenocarcinoma A549 cells and human bronchial epithelial (HBE) cells. In total, 106 differential proteins were identified and the altered expression levels of partial identified proteins were confirmed by Western blot analysis. Importantly, pathway analysis and biological validation revealed epithelial–mesenchymal transition (EMT) phenotype shift in A549 cells as compared with HBE cells. The EMT phenotype of A549 cells can be increased by self-producing TGF-β1 and significantly decreased by silencing heterogeneous nuclear ribonucleoprotein (hnRNPK) expression. As EMT has been considered as an important event during malignant tumor progression and metastasis, investigating EMT and deciphering the related pathways may lead to more efficient strategies to fight lung cancer progression. By integrating the subcellular proteomic data with EMT-related functional studies, we revealed new insights into the EMT progress of lung carcinogenesis, providing clues for further investigations on the discovery of potential therapeutic targets.

Keywords:
Cell biology / Epithelial-mesenchymal transition / Heterogeneous nuclear ribonucleoprotein K / Lung cancer / Subcellular proteomics

1 Introduction

Lung cancer represents one of the most debilitating malignancies, accounting for ~25% of all cancer deaths worldwide [1]. Regardless of subtypes, the 5-year survival rate for lung cancer is among the lowest of all cancers [2]. Because of the nodal or distant metastasis in lung cancer, the risk of recurrence cannot be reduced even complete surgical resection is performed [3]. Metastasis of lung cancer cells is one of the major reasons for the low survival rate. The progress of metastasis includes the cell detachment from primary tumor, invasion to extracellular matrix and spread

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Abbreviations: Ab, antibody; **DEP,** differentially expressed protein; **EMT,** epithelial–mesenchymal transition; **GPX1,** glutathione peroxidase 1; **HBE,** human bronchial epithelial; **hnRNP,** heterogeneous nuclear ribonucleoprotein; **KRT,** cytokeratin; **siRNA,** small interfering RNA; **VCL,** vinculin

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Colour Online: See the article online to view Figs. 3 and 8 in colour.

Parasitol Res (2011) 109:1519–1524
DOI 10.1007/s00436-011-2427-z

ORIGINAL PAPER

Synergy of the antiretroviral protease inhibitor indinavir and chloroquine against malaria parasites in vitro and in vivo

Xiaofen Li · Zhengxiang He · Lili Chen · Yayong Li · Qinyan Li · Siting Zhao · Zhu Tao · Wen Hu · Li Qin · Xiaoping Chen

Received: 4 March 2011 / Accepted: 8 April 2011 / Published online: 3 May 2011

Abstract Many malaria-endemic areas are also associated with high rates of human immunodeficiency virus (HIV) infection. An understanding of the chemotherapeutic interactions that occur during malaria and HIV co-infections is important. Our previous studies have demonstrated that some antiretroviral protease inhibitors are effective in inhibiting *Plasmodium falciparum* growth in vitro. Currently, studies examining the interactions between antiretroviral protease inhibitors and antimalarial drugs are being conducted, but the data are limited. In this study, we examined the synergistic interactions between the antiretroviral protease inhibitor indinavir and chloroquine (CQ) in chloroquine-resistant and chloroquine-sensitive malaria parasites in vitro and in vivo. In vitro, by using modified fixed-ratio isobologram method, fractional inhibitory concentrations index (FICI) was calculated to indicate the interaction between the two drugs. The results demonstrated that indinavir interacted synergistically with chloroquine against both chloroquine-sensitive *P. falciparum* clone 3D7 (mean FICI 0.784) and multidrug-resistant *P. falciparum* clone Dd2 (mean FICI 0.599). In vivo drug interactions were measured using a 4-day suppressive test in a rodent malaria model infected with *Plasmodium chabaudi*. We observed that indinavir enhanced the antimalarial activity of chloroquine against both the chloroquine-sensitive line *P. chabaudi* ASS and the chloroquine-resistant line *P. chabaudi* ASCQ. More importantly, chloroquine had a 100% clearance of asexual parasites when used in combination with indinavir at an appropriate dose ratio (10 mg/kg CQ + 1.8 g/kg indinavir) where there was no obvious toxicity. We conclude from this study that the combination of indinavir and chloroquine may become a novel antimalarial drug regimen.

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Introduction

Malaria is one of the most widespread diseases in the world, particularly in the Third World countries and especially in sub-Saharan Africa. The World Health Organization estimates that 300–500 million cases of malaria and one to two million deaths occur annually due to malaria, with most malaria-related morbidity and mortality occurring in children (Snow et al. 1999). Chloroquine (CQ) has been widely used as an antimalarial drug due to its efficacy, affordability, easy administration, and low toxicity (Ward and Bray 2001). However, the widespread emergence of drug resistance in strains of *Plasmodium falciparum* reduces the antimalarial efficacy of the drug and limits its use, which leads to the increased morbidity and mortality of malaria (Ridley 2002).

Recent studies have shown that physiologically relevant concentrations of antiretroviral protease inhibitors (APIs), which block the action of the aspartyl protease of HIV, can directly inhibit the growth of *P. falciparum* in vitro

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NOTE

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Synthesis of Quinazolin-4(3*H*)-ones via Pd(II)-Catalyzed Intramolecular C(sp^2)—H Carboxamidation of *N*-arylamidines

Bin Ma, Yong Wang, Jiangling Peng, and Qiang Zhu*

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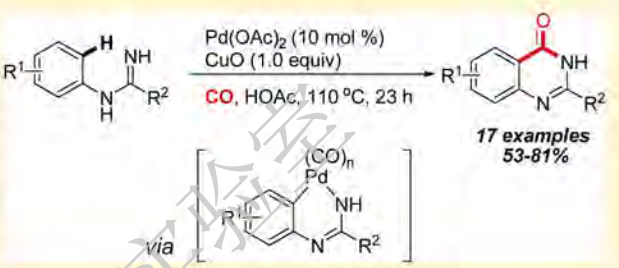
Supporting Information

ABSTRACT: An efficient synthesis of quinazolin-4(3*H*)-ones from *N*-arylamidines, through palladium-catalyzed intramolecular C(sp^2)—H carboxamidation, has been developed. The reaction, carried out in the presence of 1.0 equiv of CuO as oxidant under atmospheric pressure of CO, provides diversified 2-aryl(alkyl)-quinazolin-4(3*H*)-ones in reasonable to good yields from *N*-arylamidines, which are readily derived from anilines and nitriles. Compared with existing approaches to quinazolin-4(3*H*)-ones, the current strategy features atom-economy and step-efficiency.



Palladium-catalyzed carbonylation of alkenyl or aryl (pseudo)-halides represents a straightforward approach to carboxylic acids and their derivatives.[1] However, the requisite of using prefunctionalized (pseudo)halide substrates and the generation of environmentally unfriendly waste make alternative approaches highly desirable. Substantial achievements have been made in the area of direct functionalization of C—H bonds during the past decade.[2] Carbonylation of C—H bonds, catalyzed by transition metals under the aid of an appropriate directing group, has however attracted much less attention until very recently.[3,4] Compared with intermolecular C—H carbonylation,[3] the intramolecular fashion obviates the need for extra steps of introducing and removing the directing group which acts as an intramolecular nucleophile as well.[4] Therefore, the intramolecular C—H carbonylation is an ideal and truly atom-economical approach to lactones and lactams.[4] Herein, we would like to report a palladium-catalyzed intramolecular C(sp^2)—H carboxamidation of *N*-arylamidines, providing an efficient approach to diversified quinazolin-4(3*H*)-one derivatives.

Quinazolin-4(3*H*)-one is an important scaffold found in many natural products and synthetic drugs or drug candidates exhibiting a wide range of biological activities, including antibacterial,[5] antiinflammatory,[6] antifungal,[7] anticancer,[8] antimicrobial,[9] and antimalarial activities.[10] As a result, numerous synthetic efforts have been made for their synthesis.[11] The most widely used method is probably the condensation of 2-aminobenzoic acids or their derivatives with carboxylic acid derivatives under acidic or basic conditions (Scheme 1).[11,12] Recently, Fu developed novel cascade methods starting from 2-halobenzoic acids or 2-halobenzamides.[13] Alper and co-authors reported alternative approaches via tandem reactions involving palladium-catalyzed cyclocarbonylation of 2-iodoanilines or their derivatives as the key step.[14] We speculated that the quinazolin-4(3*H*)-one motif could also be constructed by palladium-catalyzed intramolecular C(sp^2)—H carboxamidation of simple *N*-arylamidines, which are derived readily from anilines and nitriles.[15] This strategy delivers quinazolin-4(3*H*)-ones in only two steps from commercial available chemicals, and no atoms except protons in substrates are lost during the process (Scheme 1).

The reaction conditions were optimized with *N*-phenylbenzamidine **1a** as substrate,[15,16] and the results are summarized in Table 1. Yu's conditions for the carboxylation of *o*-C—H bonds in anilides to form *N*-acylanthranilic acids, employing $Pd(OAc)_2$ (10 mol %), benzoquinone (BQ, 1.0 equiv), *p*-TsOH (0.5 equiv), HOAc/dioxane (2:1) as solvent, and atmospheric pressure of CO, were applied first (entry 1, Table 1).[3b] Gratifyingly, the desired product 2-phenylquinazolin-4(3*H*)-one **2a**, contaminated with small amount of hydroquinone, was formed in 34% yield (by NMR). To simplify the purification, inorganic oxidant $Cu(OAc)_2$ was used in place of BQ, and the yield of **2a** was improved from 52% to 59% in the absence of *p*-TsOH (entries 2—3, Table 1). Alteration of the palladium species to $PdCl_2$ or $Pd(TFA)_2$ did not improve the results. Other oxidants including $CuCl_2$, $PhI(OAc)_2$, AgOAc, $CuSO_4 \cdot 5H_2O$, and CuO were then screened (entries 6—10, Table 1), with CuO being the most effective. The selection of solvent was also crucial, identifying acetic acid as the optimum solvent (entries 11—13, Table 1).

With the optimized reaction conditions in hand, the scope of the palladium-catalyzed intramolecular C(sp^2)—H carboxamidation forming quinazolin-4(3*H*)-ones was investigated. As shown in Table 2, electron-donating groups on ortho-, meta-, and para-positions of the *N*-aryl moiety of **1** favored the reaction, providing corresponding substituted 2-phenylquinazolin-4(3*H*)-ones (**2b**—**g**) in reasonable to good yields. Notably, when two asymmetric C(sp^2)—H bonds were present in meta-substituted *N*-arylamidines **1e**,**f**, only the less hindered C—H bond was carbonylated. Unfortunately, substrates with electron-withdrawing

Received: April 8, 2011
Published: June 15, 2011

Guideline

The Chinese national guidelines on diagnosis and management of cough (December 2010)

Asthma Workgroup of Chinese Society of Respiratory Diseases (CSRD), Chinese Medical Association

Cough is a defensive physiological reflex of the human body that enables vital clearance of secretions and harmful elements from the respiratory tract; however, frequent and intense coughing may adversely impact on a patient's work, daily life and social activities. Cough is one of the most commonly observed symptoms in clinical practice arising from a wide range of aetiologies. A chronic cough may be overlooked by clinicians, particularly if reassured by the normal appearance on a chest radiograph. Furthermore, 70%–80% of these patients are readily mislabelled or diagnosed with "chronic bronchitis" or "bronchitis" in China, then typically treated with substantial but ineffective courses of antibiotics, or subjected to repeated investigations as a result of a diagnosis that is unclear, and may thus suffer more discomfort and financial burden from their disease.[1]

Increasing concerns over cough as a presenting symptom in recent years have led to a focus on clinical studies looking at the aetiologies, diagnosis and management of cough with preliminary results in China. In order to further standardize the diagnosis and treatment of acute and chronic cough, and to promote clinical and basic research in this field, the Asthma Workgroup of the Chinese Society of Respiratory Diseases (CSRD) commissioned a panel of experts in 2005 to draft the early edition of *Chinese national guidelines on diagnosis and management of cough* based on a detailed review of international[2,3] and local evidence[4,5] from clinical studies on this subject. The guideline has since played an important instructional role in domestic clinical practice in this area, and some experts and colleagues have contributed valuable comments since the first release. To include these opinions in a refined edition, and to reflect the latest progress in the research into cough, the Asthma Workgroup of the CSRD has issued this document as an update. The full-text of this revised edition basically adopts the structure of the 2005-released Guidelines, comprises eight sections, and continues to focus on chronic cough. Two sections — subacute cough and empirical treatment for chronic cough, are newly added. The diagnostic algorithm was also modified to incorporate a conception of empirical treatment, which should render the guideline more adaptable for use in community and rural hospitals.

CLASSIFICATIONS OF COUGH

Cough is typically classified into three categories depending on its duration — the acute cough (lasting less than 3 weeks), sub-acute cough (3–8 weeks) and chronic cough (more than 8 weeks), or alternatively, productive and non-productive cough depending on production of airway secretions. Different types and presentations of cough may be suggestive of distinctive causative factors. Chronic cough, in particular, may result from a very broad range of aetiologies, and can usually include two scenarios based on radiographic evidence. The one with observable abnormality on chest X-ray is often associated with underlying pathologies such as pneumonia,[6] tuberculosis[7] and lung cancer,[8] and the other with normal X-ray appearances, where cough is the major or sole symptom, represents the type of the chronic cough which is the focus in this updated edition.

HISTORY, EXAMINATION AND INVESTIGATIONS

The value of medical history of cough has been well documented elsewhere.[9] Despite the opposite remark from a single study among 88 patients,[10] meticulous assessment of the medical history and a detailed physical examination may allow for narrowing the spectrum of differential diagnoses, providing diagnostic clues to the underlying causes, or even leading to an initial diagnosis and empirical treatment. Initial clinical assessment may also guide selection of further investigations and expedite determination of underlying disorders.

History

Particular attention should be paid to the duration, timing, frequency, nature, pitch, character, predisposing or exacerbating factors, body position, and other accompanying symptoms of the cough. A review of sputum production volume, colour, odour and consistency can be of significant value to the diagnosis.

Duration of cough can help identify the category of cough (acute, subacute or chronic), and hence narrow the scope of diagnosis. The timing of cough may also be noteworthy. For instance, a cough after exercise is more common in exercise-induced asthma; nocturnal cough is frequently seen in cough-variant asthma and heart

DOI: 10.3760/cma.j.issn.0366-6999.2011.20.002
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Tumor Biol. (2011) 32:1199–1208
DOI 10.1007/s13277-011-0223-0

RESEARCH ARTICLE

The expression and clinical significance of CLIC1 and HSP27 in lung adenocarcinoma

Wei Wang · Xin Xu · Wujun Wang · Wenlong Shao · Liping Li · Weiqiang Yin · Liangchang Xiu · Mingcong Mo · Jin Zhao · Qingyu He · Jianxing He

Received: 22 May 2011 / Accepted: 8 August 2011 / Published online: 20 August 2011

Abstract The purpose of this research was to study the roles of chloride intracellular channel protein 1 (CLIC1) and heat shock protein 27 (HSP27) in the clinical pathology of lung adenocarcinoma and to explore whether the expression of CLIC1 and HSP27 can be used as independent factors for the prediction of recurrence and prognosis after radical resection of lung adenocarcinoma. One hundred and three paraffin sections of lung adenocarcinoma tissues were collected, and the expression of CLIC1 and HSP27 was detected in these tumors using immunohistochemistry. The correlation of the expression of these two proteins with clinicopathological parameters and prognosis was statistically analyzed. In the 103 samples, the expression of HSP27 and CLIC1 was strongly positive in 61 (59.2%) and 49 cases (47.6%), respectively. Statistical analysis showed that the expression level of HSP27 did not significantly correlate with the patient's age, sex, degree of tumor differentiation, T staging of tumors, and TNM staging of tumors ($p>0.05$), whereas the expression of CLIC1 did significantly correlate with T staging of tumors ($p=0.029$). Univariate analysis indicated that the patient's ECOG score, T staging, N staging, TNM staging, and CLIC1 expression correlated with prognosis ($p=0.031$, 0.001, 0.011, 0.013, and <0.001, respectively). Multivariate statistical analysis showed that age, T staging, and CLIC1 expression were independent associated factors for predicting the 5-year survival rate of patients ($p=0.026$, 0.004, and <0.001, respectively). Age, T staging, and CLIC1

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Tumor Biol. (2011) 32:1113–1121
DOI 10.1007/s13277-011-0211-4

RESEARCH ARTICLE

The expression of p33^{ING1}, p53, and autophagy-related gene Beclin1 in patients with non-small cell lung cancer

Jun Liu · Yongping Lin · Haihong Yang · Qiuhua Deng · Guoqin Chen · Jianxing He

Received: 27 June 2011 / Accepted: 5 July 2011 / Published online: 22 July 2011

Abstract The purpose of this study was to investigate the expressions of tumor inhibitor of growth (ING1) gene p33ING1, p53, and autophagy-related gene Beclin1 in human non-small cell lung cancer (NSCLC), and the correlation between their expressions with clinical pathological features and clinical significance. The research can provide new ideas and experimental evidence for early diagnosis and biotherapy for NSCLC in the future. The human NSCLC tissues and surrounding non-cancerous tissues were collected from surgical operation. The expressions of mRNA or protein of p33ING1, p53, and Beclin1 were detected by using of reverse transcription polymerase chain reaction or Western blot in these tissues. The results were used to analyze the relationships between these gene expressions with the developing of NSCLC and clinical pathological features. The expressions of mRNA or protein of p33ING1 and Beclin1 in NSCLC tissues were significantly lower than that in surrounding noncancerous tissues ($p<0.05$). The expressions of mRNA or protein of p33ING1 and Beclin1 in well- and middle-differentiated NSCLC tissues were lower than those in poor-differentiated NSCLC tissues ($p<0.05$). The expressions of mRNA or protein of p33ING1 and Beclin1 in presence of lymph nodes metastasis were lower than those in absence of lymph nodes metastasis ($p<0.05$). The expressions of mRNA or protein of p33ING1 and Beclin1 in patients of pathological stage (stages I–II) were higher than those in pathological stage (stages III–IV) ($p<0.05$). But the expression of protein of mutant-type p53 in NSCLC tissues was significantly higher than that in surrounding noncancerous tissues ($p<0.05$). The expressions of protein of mutant-type p53 in well- and middle-differentiated NSCLC tissues were higher than those in poor-differentiated NSCLC tissues ($p<0.05$). The expressions of protein of mutant-type p53 in presence of lymph nodes metastasis were higher than those in absence of lymph nodes metastasis ($p<0.05$). The expressions of protein of mutant-type p53 in patients of pathological stage (stages I–II) were lower than those in pathological stage (stages III–IV) ($p<0.05$). These expression changes of p33ING1, p53, and autophagy-related Beclin1 genes were associated with tumor cell differentiation, lymph nodes metastasis, and pathological stage of NSCLC. But these expression changes of these three genes were not associated with gender, age, size of primary carcinoma, histological type of NSCLC ($p>0.05$). The expression of mRNA of p53 and Beclin1 were correlated with p33ING1 mRNA expression in NSCLC tissues ($p<0.05$). The activity changes of tumor inhibitor of growth, autophagy, and apoptosis may be related to the emergence and the development of NSCLC. The combined detection of

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OPEN ACCESS Freely available online

PLoS one

The Golgi Localization of GOLPH2 (GP73/GOLM1) Is Determined by the Transmembrane and Cytoplamic Sequences

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Abstract

Golgi phosphoprotein 2 (GOLPH2) is a resident Golgi type-II membrane protein upregulated in liver disease. Given that GOLPH2 traffics through endosomes and can be secreted into the circulation, it is a promising serum marker for liver diseases. The structure of GOLPH2 and the functions of its different protein domains are not known. In the current study, we investigated the structural determinants for Golgi localization using a panel of GOLPH2 truncation mutants. The Golgi localization of GOLPH2 was not affected by the deletion of the C-terminal part of the protein. A truncated mutant containing the N-terminal portion (the cytoplasmic tail and transmembrane domain (TMD)) localized to the Golgi. Sequential deletion analysis of the N-terminal indicated that the TMD with a positively charged residue in the cytoplasmic N-terminal tail were sufficient to support Golgi localization. We also showed that both endogenous and secreted GOLPH2 exist as a disulfide-bonded dimer, and the coiled-coil domain was sufficient for dimerization. This structural knowledge is important for the understanding the pathogenic role of GOLPH2 in liver diseases, and the development of GOLPH2-based hepatocellular cancer diagnostic methods.

Citation: Hu L, Li L, Xie H, Gu Y, Peng T (2011) The Golgi Localization of GOLPH2 (GP73/GOLM1) Is Determined by the Transmembrane and Cytoplamic Sequences. PLoS ONE 6(11): e28207. doi:10.1371/journal.pone.0028207

Editor: Wanjin Hong, Institute of Molecular and Cell Biology, Singapore

Received September 13, 2011; **Accepted** November 3, 2011; **Published** November 29, 2011

Funding: This work was supported by National Science Foundation (309701647); National Basic Research Program (973) (Grant No. 2009CB522306). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Golgi phosphoprotein 2 (GOLPH2, also termed GP73 and GOLM1) is a type II transmembrane protein residing in the cis and medial-Golgi cisternae. GOLPH2 is predominantly expressed in the epithelial cells of many human tissues [1]. Abnormally increased expression of GOLPH2 has been reported to correlate with many diseases and viral infections. GOLPH2 overexpression has first been identified in acute giant-cell hepatitis, an uncommon form of hepatitis with a presumed viral etiology [1], and then in a variety of acute and chronic liver diseases [2,3,4,5]. Fucosylated glycosylation has also been found in three quarters of secreted GOLPH2 from hepatocellular carcinoma patients [6]. Earlier studies have indicated that both the serum level of GOLPH2 and fucos-studded GOLPH2 could be a more reliable biomarkers for the early diagnosis of liver diseases than current markers like alpha-fetoprotein [7,8].

Despite its potential as a biomarker for liver disease, knowledge on the structure and function of GOLPH2 remains very limited. Sequence analysis shows that GOLPH2 is highly conserved in vertebrates. It has a short N-terminal cytoplasmic domain followed by a transmembrane domain (TMD), and a longer C-terminal domain located in the Golgi lumen with a coiled-coil domain immediately beside the TMD [1]. To determine the possible physiological role of GOLPH2, Wright *et al.* constructed a transgenic mouse model with part of the GOLPH2 C-terminal truncated. $GP73^{tr/tr}$ mice exhibited decreased survival and severe epithelial abnormalities in the liver and kidneys, suggesting that GOLPH2 may play an important role in epithelial cell function in these organs [9].

Being a type II transmembrane protein, GOLPH2 is unlikely to be a secreted protein. A possible explanation for its secretion comes from the observations that GOLPH2 is capable of intracellular trafficking between the Golgi and plasma membrane through an endosomal pathway, as well as the existence of a conserved proprotein protease cleavage site ($R^{52}VRR^{55}$) [10]. Cleavage by cellular proprotein convertase results in the secretion of the Golgi luminal portion of GOLPH2. The level of this proteolytic cleavage is speculated to be correlated with the level of GOLPH2 abnormal overexpression [10]. However, details on the structural determinants for GOLPH2 Golgi localization and intracellular trafficking are not clear.

The subcellular localization of Golgi proteins is signal dependent. Many type II membrane proteins with a short cytoplasmic tail contain a Golgi retention signal in or around their TMDs [11,12,13]. Two different models are proposed for Golgi localization. The oligomerization model posits that the formation of oligomers prevents protein movement into transport vesicles. In contrast, the bilayer-thickness model suggests that a short TMD restricts the protein to the thinner lipid bilayer of the

International Journal for Parasitology 41 (2011) 1129–1137

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

The phenotype and function of naturally existing regulatory dendritic cells in nematode-infected mice

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ARTICLE INFO

Article history:
Received 21 February 2011
Received in revised form 21 June 2011
Accepted 22 June 2011
Available online 23 July 2011

Keywords:
Regulatory dendritic cell
Nematode parasite
Immunosuppression

ABSTRACT

Immunosuppression associated with chronic helminth infections has been documented in many studies and regulatory T (Treg) cells have been shown to mediate the nematode-induced immunosuppression, but the role of dendritic cells (DCs) in the induction of Treg cell response and immunosuppression has not yet been fully determined. We analysed the response and function of DCs in mesenteric lymph node (MLNs) of mice infected with a gastrointestinal nematode, *Heligmosomoides polygyrus*, and observed a substantial expansion of DCs in MLNs following the infection. The $CD11c^+$ DCs in MLNs of infected mice showed reduced expression of co-stimulatory molecules CD40, CD86 and MHC-II, and production of inflammatory cytokines IL-12 and IL-6. Analysis of MLN DC subsets defined by CD11c and CD45RB expression showed that the $CD11c^{low}CD45RB^{mid}$ subset increased rapidly following *H. polygyrus* infection and the $CD11c^{mid}CD45RB^{high}$ subset expanded from the third week after infection. In the co-culture of sorted DC subsets with ovalbumin-(OVA-)specific T cell receptor (TCR) transgenic $CD4^+$ T cells, $CD11c^{low}CD45RB^{mid}$ DCs induced a low proliferation response and a high level of IL-10 production in $CD4^+$ T cells, whereas $CD11c^{mid}CD45RB^{high}$ DCs induced more IFN-γ and IL-4 producing $CD4^+$ T cells. Intracellular staining revealed that $CD11c^{low}CD45RB^{mid}$ DCs promoted $CD4^+$ $Foxp3^+$ differentiations. These results indicate that nematode infections selectively induce expansion of the $CD11c^{low}CD45RB^{mid}$ regulatory DC subset that promotes development of $Foxp3^+$ and IL-10 producing Treg cells. The Treg cell responses and immunoregulatory cytokines induced by this regulatory DC subset in turn play an important role in mediation of the nematode-induced immunosuppression.

1. Introduction

Parasitic nematode infections are prevalent in many regions of the world and represent one of the most important health problems. Infections with these parasites often induce Th2-dominated immune responses which have been shown to be important for control of the infections (Urban et al., 1992). Many studies in humans and in animal models, however, also demonstrate that infections with parasitic nematodes markedly modulate the host's immune function, resulting in a suppressed immune response (Maizels et al., 2004). The nematode-induced immunosuppression is manifested as (i) a chronic course of nematode infections allowing their long-term survival (Taylor et al., 2005), (ii) impaired immunity to concurrent infection with unrelated pathogens or a reduced immune response to unrelated antigens (Su et al., 2005; Rausch et al., 2010), and (iii) protection of the host from autoimmune and allergic diseases (Schnoeller et al., 2008).

Many studies in recent years demonstrated that nematode infections are often associated with increased responses of regulatory T (Treg) cells including $Foxp3^+$ Treg cells, IL-10-producing Tr1 and TGF-β-producing Th3 $CD4^+$ T cells (Finney et al., 2007; Rausch et al., 2009, 2010). These immune regulatory cells and cytokines are the major factors in mediating the nematode-induced immunosuppression (Doetze et al., 2000; Wilson et al., 2005; Van Riet et al., 2007; Grainger et al., 2010). However, the immunological mechanisms by which the nematode parasites induce Treg responses are not fully understood.

Dendritic cells (DCs), as a professional antigen presenting cell (APC), play important roles in initiating immune responses to infection and directing the polarisation of $CD4^+$ T helper cell responses. Depending on the type of antigen or pathogen, the antigen dose and the priming conditions, DCs can induce the development of either $CD4^+$ Th1 or Th2 responses (Sher et al., 2003; Kaiko et al., 2008). DCs may also play a role in induction of immune tolerance and certain subsets of DCs, such as immature or $CD8a^-$ DCs, have been reported to preferentially promote Treg cell responses (Lutz

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0020-7519/$36.00 © 2011 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijpara.2011.06.008

RESEARCH ARTICLE

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

The Polymorphism and Haplotypes of *PIN1* Gene are Associated with the Risk of Lung Cancer in Southern and Eastern Chinese Populations

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Communicated by Christopher Mathew

Received 12 March 2011; accepted revised manuscript 5 July 2011.

Published online 17 August 2011 in Wiley Online Library (www.wiley.com/humanmutation).DOI: 10.1002/humu.21574

ABSTRACT: Peptidyl-prolyl cis/trans isomerase (PPIase), PIN1, has been found to be a critical catalyst that involves in multiple oncogenic signaling pathways. Recently, several putative functional polymorphisms of the *PIN1* gene have been identified to be associated with cancer risk. In this study, we tested the hypothesis that two common polymorphisms, c.-842G>C (rs2233678) and c.-667C>T (rs2233679), in the *PIN1* promoter are associated with risk of lung cancer. In two independent case-control studies of 1,559 lung cancer cases and 1,679 controls conducted in Southern and Eastern Chinese population, we found that compared with the most common c.-842GG genotype, the carriers of c.-842C variant genotypes (GC + CC) had a decreased risk of lung cancer (odds ratio [OR] = 0.63, 95% confidence interval [CI] = 0.51–0.78, $p = 1.13 \times 10^{-5}$). Although no association was observed between the c.-667C>T polymorphism and cancer risk, we found that the haplotype "C-C" had a greater protective effect (OR = 0.39, 95% CI = 0.23–0.67, $p = 5.03 \times 10^{-4}$). The stratification analysis showed that the protective role of c.-842C variants was more pronounced in current smokers ($p = 4.45 \times 10^{-5}$), especially in male smokers ($p = 6.71 \times 10^{-6}$) and in those who smoked more than 20 pack-years ($p = 2.30 \times 10^{-5}$) and the c.-842C variant genotypes interacted with smoking status ($P_{interaction} = 0.019$) or pack-years smoked ($P_{interaction} = 0.008$) on reducing cancer risk. Further functional assay revealed that the c.-842C variant allele had a lower transcription activity in luciferase assay and a lower DNA-binding ability with nuclear proteins, and low transcription activity in western blot assay. In conclusions, our data suggest that functional c.-842C variants and haplotype "C-C" in the *PIN1* promoter contribute to decreased risk of lung cancer by diminishing the promoter activity, which may be susceptibility biomarkers for lung cancer.
Hum Mutat 32:1299–1308, 2011. © 2011 Wiley Periodicals, Inc.

KEY WORDS: genetic susceptibility; molecular epidemiology; *PIN1*; lung cancer

Introduction

The WW (Trp–Trp) domains [Ingham et al., 2005; Zhao et al., 2009] are small conserved domains in proteins that directly bind to specific sequences in the target proteins and recruit them into signaling complexes [Lu et al., 1996; Ranganathan et al., 1997]. Exploring the function of such specific protein molecules can help us determine the transduction of oncogenic signals in cancer development [Chrencik et al., 2006; Fujii et al., 2007]. Peptidyl-prolyl cis/trans isomerase (PPIase), PIN1 (MIM# 601052), is one member of the parvulin peptidyl-prolyl isomerase (PPIases) families. With a conserved WW domain, PIN1 has a high specificity to substrate with Ser/Thr-Pro (Proline) motifs and to regulate the conformation of pro-directed phosphorylation sites [Ranganathan et al., 1997; Zhou et al., 1999] in the target proteins, such as p53 [Wulf et al., 2001], p73 [Wulf et al., 2001], cyclin D1 [Miyashita et al., 2003], c-Jun [Wulf et al., 2001], von Hippel-Lindau tumor suppressor, NIMA-related kinase 6, FCP1 [Kops et al., 2002], Tau [Zhou et al., 2000], Her-2/Neu [Ryo et al., 2002], myc [Arnold et al., 2009], axin 1 [Arnold et al., 2009], GSK-3 beta [Arnold et al., 2009; Coluccia et al., 2007], Cdc25 [Lu et al., 2002; Zhou et al., 2000], cdc2/cyclin B [Nakashima et al., 2004], β-catenin [Ryo et al., 2001], and Bcl-2 [Pathan et al., 2001], which are featured in the occurrence and metastasis of cancer. Thus, PIN1-induced conformational changes of the protein may function as a critical catalyst that potentates multiple oncogenic signaling pathways during cancer development [Ryo et al., 2003].

Aberrant over-expressions of PIN1 have been reported in lung, colon, prostate, and breast cancer tissues and cell lines [Ayala et al., 2003; Lam et al., 2008; Ryo et al., 2001]. In addition, PIN1

Additional Supporting Information may be found in the online version of this article.

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Contract grant sponsor: National Natural Scientific Foundation of China; Contract grant numbers: 30671813, 30872178, 81072366 (to J.L.), 30872142 (to W.J.), 30972540 (to B.L.), and 81001278 (to Y.Z.). Contract grant sponsor: Guangdong Provincial High Level Experts Grants; Contract grant number: 2010-79 (to J.L.). Contract grant sponsor: Guangdong Provincial Scientific Foundation; Contract grant number: 2008B060600008 (to B.L.).

Toxicology Letters 205 (2011) 320–326

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

The role of miR-506 in transformed 16HBE cells induced by anti-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide

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ARTICLE INFO

Article history:
Received 11 May 2011
Received in revised form 16 June 2011
Accepted 17 June 2011
Available online 24 June 2011

Keywords:
Anti-BPDE
Cell transformation
Human bronchial epithelial cells
MicroRNA
miR-506

ABSTRACT

Growing evidence indicates that the alteration of microRNA (miRNA) expression in tumors that is induced by chemical carcinogens plays an important role in tumor development and progression. However, the mechanism underlying miRNA involvement in lung carcinogenesis induced by anti-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (anti-BPDE) remains unclear. In our study, we used the malignant transformation of human bronchial epithelial cells (16HBE-T) induced by anti-BPDE to explore the mechanisms of human lung carcinogenesis. We found that expression of miR-506 was reduced in 16HBE-T transformed malignant human bronchial epithelial cells compared with 16HBE normal human bronchial epithelial cells. Restoration of miR-506 in 16HBE-T cells led to a decrease in cell proliferation, G0/G1 phase cell cycle arrest, as well as significantly suppressed anchorage-dependent growth *in vitro* and tumor growth inhibition in a nude mouse xenograft model. In addition, we provided novel evidence regarding the role miR-506 potentially plays in negatively regulating the protein and mRNA expression level of N-Ras in cancer cells. Together, these findings revealed that miR-506 acts as an anti-oncogenic miRNA (anti-oncomir) in malignantly transformed cells. The identification of tumor suppressive miRNAs could provide new insight into the molecular mechanisms of chemical carcinogenesis.

1. Introduction

Lung cancer is by far the leading cause of cancer death worldwide, and tobacco smoking is the primary risk factor. Cigarette smoking accounts for 80% of lung cancer cases in men and 50% in women worldwide (Thun et al., 2010). Benzo[a]pyrene (B[a]P) is one of the numerous compounds found in tobacco smoke (Sharma et al., 2008) and is also a ubiquitous environmental pollutant; it is a representative polycyclic aromatic hydrocarbon (PAH), metabolically activated by three enzymatic pathways (Jiang et al., 2007). Anti-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (anti-BPDE), the most important metabolite of B[a]P, exerts its genotoxic effects by binding covalently to DNA (Rojas et al., 2004) therefore enabling the activation or inactivation of cancer-related genes (Zhao et al., 2010). However, the molecular mechanisms involved in chemical carcinogenesis are complex and poorly understood. The discovery of microRNAs (miRNA), a class of non-coding endogenous RNAs 20–23 nucleotides in length that function as negative regulators of gene expression, has provided new opportunities in the study of the molecular mechanisms of cancer (Guarnieri and DiLeone, 2008). MiRNAs are able to negatively regulate expression of protein-coding genes through mRNA degradation or translational inhibition by binding with the 3′-untranslated regions (3′-UTR) of their mRNA targets (Engels and Hutvagner, 2006; Lim et al., 2005). Molecular alterations that target inactivation of tumor suppressor genes and activation of proto-oncogenes play a key role in the development of multistage carcinogenesis (Spandidos, 2007). Many previous studies have demonstrated that miRNA can influence the development and progression of tumors through the targeting of certain oncogenes or tumor suppressor genes.

We established 16HBE-T, a malignantly transformed cell line of the 16HBE human bronchial epithelial cell line induced by anti-BPDE, in our laboratory. We found that N-Ras expression at both the mRNA and protein levels in 16HBE-T cells were the highest among three Ras members (H-Ras, K-ras, N-Ras) and that this elevated expression plays an important role in chemically induced oncogenesis (Zhou et al., 2008). In our previous study, there were 55 significantly differentially expressed miRNAs in 16HBE-T cells identified by microarray (Shen et al., 2009). In this study, we explored the role of miRNAs in regulation of N-Ras in malignant transformation induced by anti-BPDE.

To further elucidate the potential role of miRNAs in the regulation of N-Ras, we initially utilized bioinformatic forecasts to

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0378-4274/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2011.06.022

Original Study

The Role of NF-E2-Related Factor 2 in Predicting Chemoresistance and Prognosis in Advanced Non–Small-Cell Lung Cancer

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Abstract

Background: NF-E2-related factor 2 (Nrf2) plays an important role in platinum chemoresistance by activating transcription of target genes through binding to the antioxidant response element (ARE) in gene promoters, moreover it could stimulate tumor growth in non–small-cell lung cancer (NSCLC). The objective of this study was to elucidate the correlation between Nrf2 expression and platinum-based chemotherapy response as well as the prognostic significance of Nrf2 levels. **Patients and Methods:** Immunohistochemical analysis of Nrf2 in tumor specimens was performed in a total of 60 patients with stage IIIB or IV NSCLC. **Results:** Positive staining for Nrf2 was found in nearly all cases, just at different levels. High Nrf2 expression was noted in 34 of 60 patients (56.7%). The expression of Nrf2 correlated with age (P = .014), stage (P = .017), and performance status (P = .014). The response rate of platinum-based chemotherapy in patients with < 75% positive staining was significantly higher than that in patients with 75%-100% positive staining (P = .003; r = 0.447). Furthermore, a high percentage of Nrf2 staining was the independent prognostic factor in progression survival (P = .000) analysis. **Conclusion:** We suggest that the assessment of Nrf2 expression may be useful for evaluating chemoresistance and tumor progression in patients with advanced stage NSCLC.

Clinical Lung Cancer, Vol. 12, No. 3, 166-71 © 2011 Published by Elsevier Inc.
Keywords: Advanced, Chemoresistance, Immunohistochemistry, NF-E2-related factor 2, Non–small-cell lung cancer, Prognosis

Introduction

Lung cancer is the most common malignant tumor in China.[1] Non–small-cell lung cancer (NSCLC) makes up the majority of lung cancer cases (approximately 80% of total malignancies) and has only a 15% 5-year survival rate. Most of our patients with NSCLC were found to have advanced disease (stage IIIB or IV). Advanced NSCLC is considered to be a lethal disease because the 5-year survival rate is not more than 5%.[2] Platinum-based chemotherapy with or without radiotherapy has been the standard therapy for advanced inoperable NSCLC.[3,4] Several clinical trials showed that there is no significant difference in the overall response to first-line treatment for advanced NSCLC using different agents (gemcitabine, vinorelbine, and paclitaxel) combined with platinum,[4-6] with an approximate response rate of 20%-30%. Platinum chemoresistance varies widely in the treatment of advanced NSCLC. Resistance to platinum-based chemotherapy can be intrinsic or acquired; moreover, the mechanism of resistance is multifactorial.[7]

One of the most important mechanisms of resistance to platinum is enhanced drug detoxification. Increased glutathione (GSH), glutathione-*S* transferase (GST), and other GSH-related enzymes may cause resistance by binding/inactivating platinum, reducing platinum-induced oxidative stress, or enhancing DNA repairs.[8-10] It is reported that the expression of these xenobiotic metabolism genes is increased in NSCLC.[11] NF-E2-related factor 2 (Nrf2) is a key transcription factor that is responsible for upregulation of GSH, GST, and GSH-related enzymes. Nrf2 activates transcription of these tar-

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Submitted: Jul 4, 2010; Revised: Sep 2, 2010; Accepted: Sep 13, 2010

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1525-7304/$ - see frontmatter © 2011 Published by Elsevier Inc.
doi: 10.1016/j.cllc.2011.03.012

Editorial

Towards improving the diagnosis and management of chronic cough in China

ZENG Guang-qiao, SUN Bao-qing and ZHONG Nan-shan

Cough is a common condition in clinical settings. Importantly, chronic cough without obvious abnormal chest X-ray findings accounts for about 20%–30% of respiratory clinic visits, and yields a high rate of misdiagnosis and mistreatment.[1] Up to 80% of patients with chronic cough were diagnosed with other disorders, such as "chronic bronchitis" or "chronic pharyngolaryngitis".[2,3] As many as 50% of female patients with cough may develop urinary incontinence, which can interfere seriously with their daily living, including work and school performance.[4] Along with the increasing demand for a high-quality life among Chinese people, cough as an important issue should be properly addressed by both clinicians and patients.

Research on chronic cough dates back to the late 1970s, when Irwin et al[5] pioneered the early anatomy-based protocol for diagnosing chronic cough. Studies on chronic cough were also initiated in Japan and Europe during the early 1990s. In 1998, American College of Chest Physicians (ACCP) developed the first guidelines on diagnosis and management of chronic cough,[6] and subsequently, Japanese Cough Research Society and European Respiratory Society also published their criteria in the same field.[7, 8] It was not until around 2000 that studies on the etiology, pathogenesis, and treatment of chronic cough were started in China, though, collaborated endeavors over the past years have led to encouraging advances in understanding and bedside practices of this condition.[9-11] Accordingly, the Chinese Society of Respiratory Diseases (CSRD) Asthma Workgroup released a draft National guidelines on diagnosis and management of cough (hereinafter the "Chinese guidelines")[12] in 2005, with highlights on etiology-oriented diagnosis and treatment of chronic cough. Ever since, the Chinese guidelines have been widely publicized and disseminated through scientific journals, newsletters, conferences, internet and many other channels, and have contributed to brainstorming among specialists from China, the United States and Japan. Moreover, these guidelines have remarkably raised the awareness among clinicians, especially the pulmonologists, on etiology and diagnosis of chronic cough, therefore playing a significant role in guiding clinical practices.

As a common clinical problem, chronic cough has increasingly become a concern to health providers. In many Chinese institutions, pre-clinical and clinical studies on chronic cough have been launched, induced sputum cytology introduced, and a growing body of scientific papers published to cover the epidemiology, pathogenesis and etiological diagnosis of chronic cough. In 2006, ACCP revised its guidelines on chronic cough.[13] A great deal of expert opinions and colleague comments on the draft Chinese guidelines were also well appreciated following its publication. To include these opinions and comments in a refined edition, and to reflect the latest progress in research of cough, the Asthma Workgroup of CSRD has released an update to the Chinese guidelines, which appears in this issue of *Chinese Medical Journal*.[14] Full-text of this revised edition continues to follow a philosophy of "integrated coverage, highlighted aspects and intended practicability", basically preserves the structure of the 2005-released guidelines, and has provided a systematic elucidation on diagnostic criteria and treatments for cough due to common causes. The diagnostic algorithm was developed to set priority to common rather than uncommon diseases, and simple rather than complicated tests. While subacute cough was described in a separate section, empirical treatment for chronic cough was newly added to render the guidelines more adaptable for use in community and rural hospitals. Procedures for cough provocation test were also illustrated in the annex.

Despite considerable progress in the diagnosis and management of chronic cough in China over the recent years, problems remain to be addressed. Firstly, the current understandings on etiologic distribution of chronic cough are not fully recognized to many health providers working at community or rural hospitals, who seem to be more familiar with a habitual conception of "chronic cough - chronic bronchitis/pharyngolaryngitis - antibiotics plus antitussive therapy". Secondly, in addition to the poor understanding of the etiology, misdiagnosis and mistreatment of chronic cough can also result from a lack in widespread use of specific tests. In particular the induced sputum cytology, a test diagnostically critical in chronic cough but necessitating no expensive facilities or

DOI: 10.3760/cma.j.issn.0366-6999.2011.20.001
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BMC Immunology

RESEARCH ARTICLE Open Access

Transition of tumor-associated macrophages from MHC class II^{hi} to MHC class II^{low} mediates tumor progression in mice

Benfan Wang[1,2,3], Qinyan Li[1], Li Qin[1], Siting Zhao[1], Jinyan Wang[1,4*] and Xiaoping Chen[1,2*]

Abstract

Background: Tumor-associated macrophages (TAMs) are the most abundant immune cells within the tumor stroma and play a crucial role in tumor development. Although clinical investigations indicate that high levels of macrophage (MΦ) infiltration into tumors are associated with a poor prognosis, the exact role played by TAMs during tumor development remains unclear. The present study aimed to investigate dynamic changes in TAM major histocompatibility complex (MHC) class II expression levels and to assess the effects of these changes on tumor progression.

Results: Significant inhibition of tumor growth in the murine hepatocellular carcinoma Hepa1-6 model was closely associated with partial TAM depletion. Strikingly, two distinct TAM subsets were found to coexist within the tumor microenvironment during Hepa1-6 tumor development. An MHC class II^{hi} TAM population appeared during the early phase of tumor development and was associated with tumor suppression; however, an MHC class II^{low} TAM population became increasingly predominant as the tumor progressed.

Conclusions: Tumor progression was positively correlated with increasing infiltration of the tumor tissues by MHC class II^{low} TAMs. Thus, targeting the transition of MΦ may be a novel strategy for drug development and immunotherapy.

Background

Macrophages (MΦ) represent the most abundant immune cell population in the tumor microenvironment and play a key role in tumor development [1,2]. High levels of MΦ infiltration into tumor tissues are associated with a poor prognosis; this is particularly true for hepatocellular carcinoma (HCC) [3-6]. Although a decreased number of macrophages correlates with a reduction in tumor growth in several tumor graft models [7,8], there are some exceptions. For example, depletion of Kupffer cells worsens the prognosis of tumor-bearing mice in peritoneal xenograft models because the cancer cells are able to metastasize to the liver; thus, the mice die from the increased tumor burden in the absence of MΦ [9,10]. These contradictory reports highlight the fact that little is known about the exact role of tumor-associated macrophages (TAMs) during tumor development.

MΦ are a highly heterogeneous cell population. This is because their phenotypes and diverse functions are shaped by the tumor microenvironment [11]. MΦ can be classified on the basis of two distinct activation states. Classically activated MΦ (M1), induced by IFN-γ or microbial products, produce high levels of proinflammatory cytokines (IL-12 and IL-23), express major histocompatibility complex (MHC) molecules and iNOS, and act as the primary source of anti-tumor immune cells [12-14]. In contrast, alternatively activated MΦ (M2), polarized by IL-4 or/and IL-13, secrete anti-inflammatory cytokines and are characterized by increased arginase-1 activity and the expression of Ym-1, MGL, Fizz1, and MSF [15-17]. Functionally, M2 MΦ are thought to suppress inflammation and to facilitate wound healing by promoting angiogenesis and tissue remodeling [15,18]. A recent study shows that

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SCIENCE CHINA
Life Sciences

SPECIAL TOPIC: Translational medicine in China I

December 2011 Vol.54 No.12: 1077–1080
doi: 10.1007/s11427-011-4262-y

Translational medicine: What is in a name from the perspective of Chinese clinicians?

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Received October 12, 2011; accepted November 10, 2011

Citation: Zeng G Q, Zhong N S. Translational medicine: What is in a name from the perspective of Chinese clinicians?. Sci China Life Sci, 2011, 54: 1077−1080, doi: 10.1007/s11427-011-4262-y

Translational medicine is an emerging medical practice that involves multidisciplinary collaborations to bridge between basic sciences and clinical use, aiming at enhancing patient care and preventive measures. As it suggests for most, basic scientists in particular, the term "translational medicine" may literally imply a uni-directional endeavor to use data or findings in basic research for development of novel therapies or medical procedures, and then test them in humans. This is not always the case, because what is found in laboratories does not fully mean what should happen in a real-world human body. More efforts need to be focused on feedback understandings of how humans react to the treatment. In fact, the one-way concept no longer suffices, and a two-way feature of translational medicine has been proposed elsewhere [1]: from bench to bedside and from bedside to bench.

Although starting late, translational medicine as an inter-disciplinary science is developing rapidly and widely in China. Sustainability of such development entails close collaboration between persons on laboratory benches and those at the bedside. Unfortunately, the paradigm between basic and clinical science has often put these two disciplines at odds with each other [2], and communications between basic and clinical scientists is rare and sporadic [3]. The ultimate goal of this science behooves us to have a better look at what translational medicine means to Chinese clinicians.

For the part of Chinese clinicians, translational medicine may be interpreted in several directions (Figure 1).

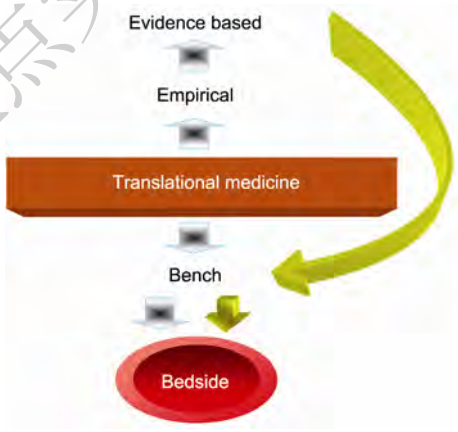

Figure 1 On the part of Chinese clinicians, the routes of translation should ideally be set as (i) from bench to bedside; (ii) from empirical to evidence based, and (iii) from bedside to bench then back to bedside.

1 From bench to bedside

"From bench to bedside" represents the traditional one-way modality of translational medicine, in which, clinicians are recipients of overwhelming data surging out of the basic science community. Since China is a developing country with a large population of patients but very limited financial resources, our clinicians need to translate laboratory findings into simpler, cheaper and effective products or measures with less adverse effects for purposes of clinical diagnosis and management.

This rationale can be well illustrated with our previous

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Editorial

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Cough is a common condition in clinical settings. Importantly, chronic cough without obvious abnormal chest X-ray findings accounts for about 20%–30% of respiratory clinic visits, and yields a high rate of misdiagnosis and mistreatment.[1] Up to 80% of patients with chronic cough were diagnosed with other disorders, such as "chronic bronchitis" or "chronic pharyngolaryngitis".[2,3] As many as 50% of female patients with cough may develop urinary incontinence, which can interfere seriously with their daily living, including work and school performance.[4] Along with the increasing demand for a high-quality life among Chinese people, cough as an important issue should be properly addressed by both clinicians and patients.

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第一作者类论文

ORIGINAL ARTICLE AIRWAY DISEASES

Indoor allergen levels in Guangzhou city, southern China

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To cite this article: Zhang C, Gjesing B, Lai X, Li J, Spangfort MD, Zhong N. Indoor allergen levels in Guangzhou city, southern China. *Allergy* 2011; **66**: 186–191.

Keywords
cockroach; exposure; house dust mite; indoor allergens; risk factors.

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Accepted for publication 9 July 2010

DOI:10.1111/j.1398-9995.2010.02465.x

Edited by: Hans-Uwe Simon

Abstract

Background: High levels of sensitization to house dust mites have been observed in Chinese allergic patients. This study has measured levels and distributions of mite and cockroach allergens in household dust in Guangzhou. Influences of home characteristics and seasonal changes on allergen levels were also investigated.
Methods: Dust samples were collected from bedding and living room from households in Guangzhou. Major allergens from *Dermatophagoides pteronyssinus*, *D. farinae*, *D. microceras*, *Blomia tropicalis* and cockroach allergens were measured by ELISA. Home characteristics were obtained from a questionnaire.
Results: Four hundred and four dust samples were collected from 107 homes during October 2006 to November 2007. House dust mite allergen levels were detectable in 99% of the bedding samples. Der f 1 levels were significantly higher than Der p 1 levels. High levels of mite allergens (> 10 μg/g) were observed in 88% of all the bedding samples. Cockroach allergens were detected in 93% of households and were higher in living room samples than in bedding samples. Blo t 5 and Der m 1 could not be detected in the dust samples. Having fabric furniture was a predictor of high allergen levels. Der f 1 levels were higher in summer time than in winter time. Cockroach allergens were higher in winter time than in summer time.
Conclusion: In Guangzhou, Der f 1 is the predominant mite allergen in dust with very high levels in bedding. Cockroach allergens are also common.

House dust mites are very important sources of indoor allergens worldwide, including China. Exposure and sensitization to house dust mite allergens have been associated with development of asthma and other allergic diseases (1, 2). Recently, China Alliance of Research on Respiratory Allergic Disease (CARRAD) showed that the prevalence of positive skin responses (SPT) among more than 6000 patients with asthma and/or allergic rhinitis from China were highest for *Dermatophagoides farinae*, *D. pteronyssinus*, *Blomia tropicalis* and cockroach species *Periplaneta americana* and *Blattella germanica* (3). The SPT prevalence (N = 668) for Guangzhou city were 64% for *D. farinae* and *D. pteronyssinus*, 43% for *B. tropicalis*, 18% for *P. americana* and 10% for *B. germanica*.

Allergen exposure can be indicated by the number of mites or the levels of their allergens detected in household dust. Mite fauna has been investigated in Guangzhou city showing that *D. pteronyssinus* and *D. farinae* are most frequently found (4, 5), but there is no study that has measured indoor allergens in dust samples from this city.

The aim of this study was to determine the levels and distribution of mite and cockroach indoor allergens in household dust from bedding and living room in Guangzhou city. In addition, the influences of home characteristics and seasonal changes on the allergen levels were investigated.

Material and methods

Subjects

The homes of 107 subjects were included in this study (62 subjects were allergic outpatients visiting Guangzhou Institute for Respiratory Diseases and 45 were subjects with no clinical history or symptoms of allergy). All subjects lived in Guangzhou city, southern China. Written informed consent was obtained from participants or their parents.

Dust collection

Two dust samples per visit, one from beddings and one from living room, were collected in each home on two occasions with 6 months interval. Subjects had been living in their homes for at least 1 year before dust collection. Participants were asked not to replace their bedclothes for 2 weeks prior

Carcinogenesis vol.32 no.5 pp.713–722, 2011
doi:10.1093/carcin/bgr035
Advance Access publication February 24, 2011

Low-level expression of *let-7a* in gastric cancer and its involvement in tumorigenesis by targeting *RAB40C*

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Gastric cancer is the fourth most common cancer and the second leading cause of cancer mortality worldwide but the underlying molecular mechanism is not entirely clear. The objective of this study was to explore the role of *let-7a* microRNA (miRNA) in gastric tumorigenesis and the possible correlation between *RAB40C* and *let-7a* miRNA in gastric cancer. We found that expression of *let-7a* is reduced in human gastric cancer tissues and cell lines and there was a significant correlation between the level of *let-7a* expression and the stage of differentiation. Overexpression of *let-7a* resulted in a decrease in cell proliferation and G_1 arrest, significantly suppressed anchorage-dependent growth *in vitro* and the tumorigenicity of gastric cancer cells in a nude mouse xenograft model. Furthermore, we demonstrated that *RAB40C* is regulated directly by *let-7a* and plays an essential role as a mediator of the biological effects of *let-7a* in gastric tumorigenesis. This study revealed that *let-7a* is significant in suppressing gastric cancer growth *in vivo* and *in vitro* and provided the first evidence that *RAB40C* is negatively regulated by *let-7a* at the posttranscriptional level via binding to the 3′-untranslated region of *RAB40C* messenger RNA in gastric cancer. The results of this study suggest that *let-7a* and *RAB40C* are potentially useful targets for gastric cancer diagnosis and therapy.

Introduction

Gastric cancer is the fourth most common cancer and the second leading cause of cancer mortality worldwide despite a decreasing incidence in recent decades (1). It remains an important public health burden worldwide, especially in developing countries. In China, gastric cancer has the highest mortality among all cancers and the overall mortality rate has increased steadily in the past 20 years (2). However, the molecular mechanisms involved in gastric cancer are diverse, complex and not fully understood.

New opportunities in the study of cancer molecular mechanisms have been provided by the discovery of microRNAs (miRNAs), a class of short non-coding endogenous RNAs that function as negative regulators of gene expression (3). As the major endogenous triggers for posttranscriptional silencing, miRNAs can negatively regulate the expression of a protein-coding gene by binding with the 3′-untranslated regions (3′-UTRs) of their messenger RNA (mRNA) targets and then repressing expression of the target gene through mRNA degradation or translational inhibition (4,5). miRNAs are predicted to target more than one-third of human genes and each miRNA can control hundreds of target genes (6). Moreover, miRNAs have been demonstrated to be evolutionarily conserved and to perform regulatory functions in numerous biological processes, including developmental timing, cell proliferation, apoptosis, metabolism, cell differentiation and morphogenesis (7–9).

Recently acquired evidence demonstrates that miRNAs can be regulators in carcinogenesis. Calin *et al.* (10) showed that >50% of the known mature human miRNA genes are located in cancer-associated genomic regions or in fragile sites, suggesting that miRNAs might have an important role in the pathogenesis of human cancers. Moreover, different cancer types have distinct miRNA expression profiles, and an increasing number of miRNAs have been suggested to have important roles in tumor progression or in tumor suppression (11–13). Increased expressions of some miRNAs, such as *miR-21* and *miR-27a*, have been found to play crucial roles in gastric tumors (14,15). In addition, the miR-106b-25 cluster, which is upregulated in human gastric tumors, is involved in the posttranscriptional regulation of transcription factor E2F1 (16) and miR-15b and miR-16 modulate multi-drug resistance by targeting B-cell lymphoma/leukmia-2 (BCL2) in human gastric cancer cells (17). In contrast, *miR-9*, *miR-141*, *miR-143*, *miR-145*, *miR-433* and *miR-451* are downregulated in gastric cancer and these miRNAs act as anti-oncogenic miRNAs with a significant growth inhibitory effect on gastric cancer (18–21).

Among all human cancer-related miRNAs, the *let-7* family has attracted the most interest because its family members have been noted to express aberrantly in human cancers (22,23). The family was discovered initially in *Caenorhabditis elegans* and is currently one of the most important members of the miRNA family. The *let-7* family consists of 11 very closely related genes and many human *let-7* genes map to regions that are altered or deleted in human tumors, indicating that these genes might function as tumor suppressors (22). Moreover, when overexpressed in colon cancer cells, *let-7* miRNA leads to growth proliferation associated with a reduced level of *RAS* protein (24). *let-7a* is downregulated in Burkitt's lymphoma and it has been shown to be an anticancer miRNA that repressed *C-MYC* expression at the translational level (25). Recently, the implication of *let-7* in carcinogenesis has been extended to the repression of high-mobility group A2, thus preventing oncogenic transformation in many tumors (26,27). These findings suggest that *let-7* miRNAs participate actively in tumorigenic processes and the targets involved in the regulation of *let-7* miRNAs have been associated with various tumorigenic processes in addition to the miRNAs themselves. However, the data for the relationship between gastric carcinogenesis and the expression of *let-7a* miRNA are very limited. Evidence collected to date shows *let-7a* was linked to the modulation of different target genes, the most well-known being the *RAS* family. The *RAS* proteins function as the critical molecular switch for various signaling pathways controlling the diverse biological processes. *RAB40C* is a member of the *RAS* family, which plays important roles in tumorigenesis. With the help of a bioinformatic analysis, we found *RAB40C* contained the *let-7a* binding site and was evolutionarily conserved across 10 species. To our knowledge, there is no report of work investigating the role of *let-7a* or a possible correlation between *RAB40C* and *let-7a* miRNA in gastric cancers.

Abbreviations: CCK-8, cell counting kit-8; DMEM, Dulbecco's modified Eagle's medium; inhibitor NC, inhibitor non-specific control miRNA; mimic NC, mimic non-specific control miRNA; mRNA, messenger RNA; miRNA, microRNA; PCR, polymerase chain reaction; RT, reverse transcription; siRNA, small interfering RNA; 3′-UTR, 3′-untranslated region.

[†]These authors contributed equally to this work.

Effect of plasmid-mediated RNA interference targeting telomerase reverse transcriptase on lung cancer cells

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Received June 21, 2011; Accepted July 22, 2011

DOI: 10.3892/or.2011.1455

Abstract. In the present study, a plasmid-mediated siRNA interference vector targeting the hTERT gene was constructed and stably transfected into H1299 lung cancer cells. Using real-time quantitative fluorescent PCR technology, Western blotting and flow cytometry-based cell cycle profiling, the silencing effect of this vector and its inhibitory effect on proliferation in lung cancer cells were explored. Based upon the results of our previous study, a pair of siRNA sequences was selected, and a DNA template primer was designed and synthesized. After cloning of the template primer into the promoter of the pGenesil-1.1 expression vector, the constructed interference vector was validated using enzyme digestion and gene sequencing. The recombinant interference vector and empty vector were separately transfected into H1299 lung cancer cells with cationic liposomes, and stable monoclonally transfected cells were obtained after selection with G418. After stable transfection, hTERT mRNA and protein expression levels were detected using real-time RT-PCR technology and Western blotting. Using the MTT method and a colony formation assay, the growth and proliferation of the stably transfected lung cancer cells were determined. Changes in the cell cycle profile of the stably transfected lung cancer cells were detected using flow cytometry. An interference vector targeting the hTERT gene (pGenesil.1-hTERT) was successfully constructed. Enzyme digestion and gene sequencing confirmed that the sequence insertion met the criteria of the design. After transfection of H1299 cells with pGenesil.1-hTERT or an empty vector, the stably transfected monoclonal cell lines H1299-pGenesil.1-hTERT and H1299-pGenesil.1 were obtained. Compared to the control cells transfected with the empty vector, the H1299-pGenesil.1-hTERT cells had significantly lower mRNA expression of hTERT (93.97±0.83% inhibition, with $P<0.001$). The protein expression of hTERT in H1299-pGenesil.1-hTERT cells was significantly lower compared to that in H1299-pGenesil.1 cells. The rate of proliferation of H1299-pGenesil.1-hTERT cells was lower compared to that of H1299-pGenesil.1 lung cancer cells. In H1299-pGenesil.1-hTERT cells, the number of cells in the G1 phase increased by 18.3% ($P<0.05$) compared to the control group; the number of cells in the S and G2 phases decreased by 10.4 and 7.9%, respectively ($P<0.05$). A recombinant plasmid that interfered with the expression of the hTERT target gene was successfully constructed. Upon transfection of the recombinant interference plasmid into H1299 lung cancer cells, hTERT mRNA and protein expression were down-regulated effectively, telomerase activity and cell proliferation were inhibited, and the cell cycle profile was altered.

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Key words: lung cancer, human telomerase reverse transcriptase gene, RNA interference

Introduction

RNA interference is a gene silencing technology that was developed by Fire *et al* (1) during their investigation of the mechanism of gene silencing in the nematode *C. elegans* in 1998. When an endogenous mRNA sequence was inserted into a homologous double-stranded RNA in cells, specific degradation of that mRNA occurred, leading to the phenomenon of gene silencing. This gene silencing effect occurred at the post-transcriptional level and is therefore, also known as post-transcriptional gene silencing (PTGS).

The functional mechanism underlying the action of RNAi is a multi-step process that involves many factors induced by double-stranded RNA (dsRNA), is a part of post-transcriptional gene regulation, and requires the participation of ATP. RNA interference includes initiation and effector steps. At the initiation stage, dsRNA is cleaved by the endonuclease RNase III (also known as Dicer in *Drosophila*) into 21 to 23 nucleotide fragments based on the small interfering RNA (siRNA). At the effector stage, the siRNA binds to Dicer-l and Dicer-2 (members of the Dicer family), RNAi-specific enzymes (such

RNAi targeting of hTERT gene expression induces apoptosis and inhibits the proliferation of lung cancer cells

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Received January 21, 2011; Accepted July 28, 2011

DOI: 10.3892/ol.2011.388

Abstract. The present study aimed to investigate the effects of RNAi-mediated reduction in human telomerase reverse transcriptase (hTERT) expression on apoptosis and lung cancer cell proliferation. A number of cell lines, including 95D, were used. hTERT mRNA levels were detected, and the RNA concentration was calculated. MTT assay was used to detect the inhibition of cell proliferation. The siRNA with the highest suppression rate, siRNA-1, was transfected into 95D cells at three different concentrations (50, 80 and 100 nmol/l). The levels of hTERT mRNA in cells transfected with 50 nmol/l siRNA-1 were not significantly different from those of the negative control-transfected cells (P>0.05), whereas both 80 and 100 nmol/l siRNA-1 showed significant reductions in hTERT mRNA compared to the negative control cells (P<0.01). hTERT levels in the 80- and 100-nmol/l groups were not significantly different (P>0.05). Compared with the control cells, cells transfected with 50, 80 or 100 nmol/l siRNA-1 showed higher fractions of apoptotic cells 48 h post-transfection (P<0.01), although the apoptotic fraction in cells transfected with 50 nmol/l siRNA-1 was not significantly different compared to that in cells transfected with negative control siRNAs (P>0.05). Moreover, the 80- and 100-nmol/l-transfected cells showed significantly increased apoptotic indices (P<0.01). MTT results indicated a time-dependent inhibition of siRNA-1-transfected cell proliferation starting at 12 h and lasting through 48 h post-transfection; the inhibition was attenuated by 72 h post-transfection. The high levels of hTERT mRNA in all human lung cancer cell lines tested suggest that telomerase plays a role in lung carcinogenesis, and this hypothesis was strengthened by the data showing that the siRNA-mediated reduction in hTERT mRNA caused apoptosis and an inhibition of the proliferation of lung cancer cells.

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Key words: lung cancer, human telomerase reverse transcriptase, siRNA-1, apoptosis, proliferation

Introduction

Telomeres are disposable DNA sequences that preserve chromosomal integrity during mitosis. Human telomerase is a ribonucleoprotein comprising human telomerase RNA (hTR) and related proteins, which prevents telomere degradation, loss, rearrangement or end-to-end fusion (1). One of these related proteins, human telomerase reverse transcriptase (hTERT), uses hTR as a template to continuously synthesize telomeric DNA sequences at the ends of chromosomes. Although telomerase activity in normal cells is only detected in cells with proliferative potential, such as germ line and hematopoietic cells, as well as activated lymphocytes (2), the majority of malignant carcinoma cells exhibit telomerase activity. For example, telomerase activity is detected in up to 80% of non-small cell lung carcinoma cells (3,4), suggesting that the inhibition of telomerase activity in tumor cells blocks telomeric repair, leading to a gradual reduction in telomere length during each round of replication and subsequent cell senescence and death. Therefore, telomerase has received much attention in the investigation into cancer treatment strategies. Studies (5,6) have confirmed that telomerase inhibition by approaches including the use of exogenous antisense oligonucleotides are capable of inhibiting intracellular telomerase activity and therefore blocking cell growth and inducing apoptosis.

In the present study, 95D giant-cell lung carcinoma cells, which exhibit a high hTERT expression, were used for RNAi experiments, wherein siRNAs specifically targeting hTERT mRNA were transfected into cells and the effects of hTERT reduction on tumor cell growth and proliferation were analyzed. This study provides experimental evidence for the application of RNAi technology to the treatment of lung cancer and also provides new data on the role of telomerase in lung cancer.

Materials and methods

Materials. The following cells were used in this study: L78 and NCI-H520 human squamous cell lung cancer cells, A549

Short-Course Chemotherapy with TMC207 and Rifapentine in a Murine Model of Latent Tuberculosis Infection

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***Rationale*: Multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) is an emerging global health threat. Proper management of close contacts of infectious patients is increasingly important. However, no evidence-based recommendations for treating latent TB infection (LTBI) after MDR/XDR-TB exposure (DR-LTBI) exist. An ultrashort regimen for LTBI caused by drug-susceptible strains (DS-LTBI) is also desirable. TMC207 has bactericidal and sterilizing activity in animal models of TB and improves the activity of current MDR-TB therapy in patients.**

***Objectives*: The objective of this study was to determine whether TMC207 might enable short-course treatment of DR-LTBI and ultrashort treatment of DS-LTBI.**

***Methods*: Using an established experimental model of LTBI chemotherapy in which mice are aerosol-immunized with a recombinant bacillus Calmette-Guérin vaccine before low-dose aerosol infection with *Mycobacterium tuberculosis*, the efficacy of TMC207 alone and in combination with rifapentine was compared with currently recommended control regimens as well as once-weekly rifapentine + isoniazid and daily rifapentine ± isoniazid.**

***Measurements*: Outcomes included monthly lung colony-forming unit counts and relapse rates.**

***Main Results*: Lung colony-forming unit counts were stable at about 3.75 $\log_{10}$ for up to 7.5 months postinfection in untreated mice. Rifamycin-containing regimens were superior to isoniazid monotherapy. TMC207 exhibited sterilizing activity at least as strong as that of rifampin alone and similar to that of rifampin + isoniazid, but daily rifapentine +/− isoniazid was superior to TMC207. Addition of TMC207 to rifapentine did not improve the sterilizing activity of rifapentine in this model.**

***Conclusions*: TMC207 has substantial sterilizing activity and may enable treatment of DR-LTBI in 3–4 months.**

Keywords: bacillus Calmette-Guérin; mouse; isoniazid; rifampin; pyrazinamide

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Close contacts of patients with multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) have a significant risk of developing MDR/XDR-TB themselves. Current recommendations for treating latent TB infection among close contacts call for 6–12 months of treatment with pyrazinamide plus a fluoroquinolone or ethambutol. A shorter regimen active against all strains of MDR/XDR-TB is highly desirable. TMC207 has strong bactericidal activity in animal models of TB and improves existing therapies for MDR-TB, but the sterilizing activity of TMC207 alone has not been examined.

What This Study Adds to the Field

This study provides further validation of a paucibacillary murine model of latent TB infection (LTBI) treatment and demonstrates that TMC207 has sterilizing activity as great as that of currently recommended rifamycin-containing short-course regimens for drug-susceptible LTBI and, therefore, may effectively treat LTBI after MDR/XDR-TB exposure in 3–4 months.

Multidrug-resistant and extensively drug-resistant tuberculosis (MDR/XDR-TB) is an emerging crisis that threatens to undermine efforts to control TB (1). For every case of MDR/XDR-TB, there are close contacts who carry a substantial risk of developing active MDR/XDR-TB (2). Current guidelines recommend that close contacts of patients with MDR/XDR-TB should be carefully monitored for at least 2 years to facilitate prompt and appropriate treatment if they develop active TB (3). Treatment of latent TB infection (LTBI) among close contacts of patients with MDR/XDR-TB may prevent the development of active disease, with its attendant morbidity and cost, and help to control the spread of drug-resistant strains. However, clear guidance on the treatment of LTBI among contacts of patients with MDR/XDR-TB (DR-LTBI) is lacking. Current recommendations for empirical treatment of DR-LTBI call for pyrazinamide (PZA) combined with either ethambutol or a fluoroquinolone for 6 to 12 months (3). However, there are few data to support these recommendations and, in some outbreak settings, combinations of PZA with ofloxacin or levofloxacin have been associated with treatment-limiting hepatotoxicity (4–7). A simple and safe short-course regimen of proven efficacy to treat DR-LTBI would be an important advance.

Ultrashort regimens to treat LTBI due to drug-susceptible strains of *Mycobacterium tuberculosis* are also desirable. Using an improved paucibacillary mouse model of LTBI treatment, we showed that daily treatment with rifapentine (RPT) was more effective than rifampin (RIF) plus isoniazid (INH), and as effective as RIF + PZA (8), suggesting that daily RPT-based regimens may be capable of treating LTBI in 2 months or less.

TMC207 (TMC, J) is a novel diarylquinoline ATP synthase inhibitor with potent *in vitro* activity against *M. tuberculosis*, including strains resistant to commonly used first- and second-

(*Received in original form March 6, 2011; accepted in final form May 25, 2011*)

Supported by National Institutes of Health contract N01-AI-40007.

Author contributions: Conception and design: T.Z., K.A., E.N.; acquisition and analysis of data: T.Z., S.L., K.W., E.N.; interpretation of data and drafting of manuscript: T.Z., E.N.; reviewing the manuscript for important intellectual content: T.Z., K.A., E.N.

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Am J Respir Crit Care Med Vol 184. pp 732–737, 2011
Originally Published in Press as DOI: 10.1164/rccm.201103-0397OC on June 9, 2011
Internet address: www.atsjournals.org

参与类论文

nature genetics

A genome-wide association study identifies two new lung cancer susceptibility loci at 13q12.12 and 22q12.2 in Han Chinese

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Lung cancer is the leading cause of cancer-related deaths worldwide. To identify genetic factors that modify the risk of lung cancer in individuals of Chinese ancestry, we performed a genome-wide association scan in 5,408 subjects (2,331 individuals with lung cancer (cases) and 3,077 controls) followed by a two-stage validation among 12,722 subjects (6,313 cases and 6,409 controls). The combined analyses identified six well-replicated SNPs with independent effects and significant lung cancer associations ($P < 5.0 \times 10^{-8}$) located in *TP63* (rs4488809 at 3q28, $P = 7.2 \times 10^{-26}$), *TERT-CLPTM1L* (rs465498 and rs2736100 at 5p15.33, $P = 1.2 \times 10^{-20}$ and $P = 1.0 \times 10^{-27}$, respectively), *MIPEP-TNFRSF19* (rs753955 at 13q12.12, $P = 1.5 \times 10^{-12}$) and *MTMR3-HORMAD2-LIF* (rs17728461 and rs36600 at 22q12.2, $P = 1.1 \times 10^{-11}$ and $P = 6.2 \times 10^{-13}$, respectively). Two of these loci (13q12.12 and 22q12.2) were newly identified in the Chinese population. These results suggest that genetic variants in 3q28, 5p15.33, 13q12.12 and 22q12.2 may contribute to the susceptibility of lung cancer in Han Chinese.

In China, the incidence and mortality rates of lung cancer have been increasing rapidly in the last three decades, primarily because of a continuous increase in tobacco consumption[1]. Although lung cancer is tobacco related, genetic factors also play an important role in lung carcinogenesis. Genomic regions at chromosomes 15q25.1 (*CHRNA5*, *CHRNA3* and *CHRNA4*)[2–4], 5p15.33 (*TERT-CLPTM1L*)[5,6] and 6p21.33 (*BAT3-MSH5*)[5] have been identified to be associated with susceptibility to lung cancer by genome-wide association studies (GWAS) in populations of European descent. However, studies have also shown the presence of genetic heterogeneity in lung cancer susceptibility between populations of European descent and Asians[2–4,7–10]. For example, three SNPs, rs8034191, rs1051730, and rs16969968 at 15q25.1, were consistently found to be associated with lung cancer risk in populations of European descent[2–4,7,8] but were not replicated in Asians, and the allelic frequencies of these three risk SNPs were very low in both the Chinese and Japanese populations (minor allele frequency (MAF) < 0.05)[9,10]. Likewise, the variants at chromosome 6p21.33, rs3117582 of *BAT3* and rs3131379 of *MSH5*, which were previously reported[5], were not polymorphic among Asians.

It is biologically conceivable that the same susceptibility variant for lung cancer may be implicated across different populations. However, it is also possible that variants identified in populations of European descent might not be applicable among Asians because of underlying

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Received 10 January; accepted 6 June; published online 3 July 2011; doi:10.1038/ng.875

Brief report

Allergen micro-array detection of specific IgE-reactivity in Chinese allergy patients

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Keywords: *specific immunoglobulin E; micro-array; Dermatophagoides pteronyssinus; Dermatophagoides farinae; cross-reactivity*

Background Allergen micro-arrays are powerful tools for screening of serum IgE-reactivity. In this study allergen micro-arrays were used to identify dominating IgE-binding allergens and cross-reactivity patterns among selected Chinese allergy patients.

Methods The study was conducted using patient sera from the cities of Guangzhou, Nanjing, Chengdu and Shenyang. In total 100 sera with *Dermatophagoides pteronyssinus* (Der p) specific IgE-levels higher than 50 kU/L were selected for testing against 103 individual allergens.

Results Among 100 selected patients, 95% showed IgE-reactivity towards house-dust mite allergens *Dermatophagoides farinae* (Der f) 1, Der f 2 and Der p 2 and 94% were IgE positive against Der p 1, and 60% of sera contained IgE reacting against allergen *Euroglyphus maynei* (Eur m) 2. IgE against cat allergen, *Felisdomesticus* (Fel d) 1, was seen in 20%. Only 2% showed specific IgE-reactivity to Der p 10, a panallergen belonging to the tropomyosin family. Serum IgE-reactivity towards other allergens was in general low. IgE-reactivity against pollen allergens showed geographic differences.

Conclusions This study clearly confirms that group 1 and group 2 are major allergens of house dust mites. These selected house-dust mite allergy patients are close to being mono-sensitized. Der p 10 is not an important allergen for cross-reactivity. Specific IgE-sensitization towards pollen allergens is low in southern China compared to other regions. The prevalence of food and stinging insect allergens known to give rise to IgE-mediated cross-reactivity is 2% or less.

Chin Med J 2011;124(24):4350-4354

Allergen micro-arrays are powerful tools for screening of serum IgE-reactivity against a large number of purified single allergens using a small amount of sera. In populations where the specific IgE and IgE-mediated cross-reactivity patterns need to be defined, allergen micro-arrays are convenient to use since a very large number of allergens representing inhaled, food and stinging insect allergenic sources can be arrayed together with allergens known to induce serum IgE-mediated cross-reactivity. Thus, allergen micro-arrays can help to identify dominating allergenic sources and allergens and indicate patterns of poly-sensitizations and IgE-mediated cross-reactivity among various allergenic sources. Several studies have also suggested that allergen micro-arrays may play an important role in future specific allergy diagnosis in combination with clinical history and *in vivo* testing.[1-4]

In China, house-dust mites of the species *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) have been identified to be the dominating indoor allergenic sources with major allergens belonging to group 1 and group 2; following the nomenclature of the World Health Organization-International Union of Immunological Societies (WHO-IUIS) (*www.allergen.org*).[5,6] However, due to lack of specific well-characterized reagents, it is not known if these patients are mono-sensitized to Der p/Der f or also are they sensitive to other allergens; e.g. pollens and foods. Sensitization against cockroaches, animal dander, moulds and storage mites have been shown to have a relatively high prevalence based on skin prick test (SPT) reactivity in Chinese allergy patients.[6] Several pollen sources have also been suggested to cause allergic sensitization in China; including mugwort, ragweed, and Japanese hops.[6-9] However, a complete picture of the allergenic landscape in China is probably not yet fully

DOI: 10.3760/cma.j.issn.0366-6999.2011.24.039
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JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Antimalarial β-Carboline and Indolactam Alkaloids from *Marinactinospora thermotolerans*, a Deep Sea Isolate

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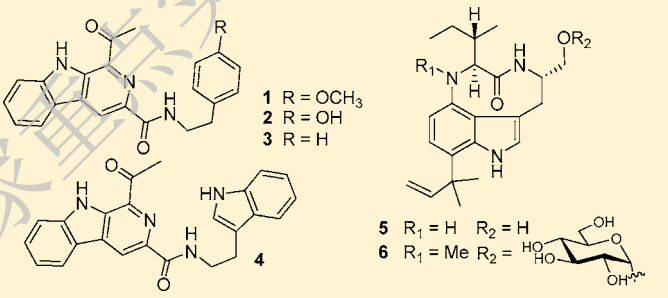
Supporting Information

ABSTRACT: Four new β-carboline alkaloids, designated marinacarbolines A–D (**1**–**4**), two new indolactam alkaloids, 13-*N*-demethyl-methylpendolmycin (**5**) and methylpendolmycin-14-*O*-α-glucoside (**6**), and the three known compounds 1-acetyl-β-carboline (**7**), methylpendolmycin (**8**), and pendolmycin (**9**) were obtained from the fermentation broth of *Marinactinospora thermotolerans* SCSIO 00652, a new actinomycete belonging to the family Nocardiopsaceae. Their structures were elucidated by extensive MS and 1D and 2D NMR spectroscopic data analyses. The structure of compound **1** was further confirmed by single-crystal X-ray crystallography. The new compounds **1**–**6** were inactive against a panel of eight tumor cell lines (IC_{50} > 50 μM) but exhibited antiplasmodial activities against *Plasmodium falciparum* lines 3D7 and Dd2, with IC_{50} values ranging from 1.92 to 36.03 μM.



Actinomycetes are important economical microorganisms and play a leading role in the production of bioactive natural products.[1] In the past 10 years, new compounds originating from marine actinomycetes have surpassed the production by their terrestrial counterparts, and marine actinomycetes are considered an exciting new resource for drug discovery.[2,3] It has been demonstrated that new genera or species of marine actinomycetes are capable of producing novel chemotypes. For example, the antitumor antibiotic salinosporamides from the new genus *Salinispora*,[4] antibacterial abyssomicins from the new species *Verrucosispora* sp. AB-18-032,[5,6] and the antitumor and antibacterial marinomycins from the new genus *Marinispora*[7] are several structure classes with novel scaffolds. These discoveries inspired us to search for new actinomycetes from the South China Sea and to explore their novel, biologically active secondary metabolites. Recently, we reported two new genera and one new species of marine actinomycetes from the South China Sea marine sediments.[8−10] Among them, the actinomycete strain SCSIO 00652 was indentified to be a novel genus, designated *Marinactinospora thermotolerans*, belonging to the family Nocardiopsaceae.[8] This strain was isolated from a sediment sample collected at a depth of 3865 m. A chemical investigation of this strain resulted in the isolation of nine alkaloids, including four new β-carboline alkaloids, designated marinacarbolines A–D (**1**–**4**), two new indolactam alkaloids, 13-*N*-demethyl-methylpendolmycin (**5**) and methylpendolmycin-14-*O*-α-glucoside (**6**), and the three known analogues 1-acetyl-β-carboline (**7**), methylpendolmycin (**8**), and pendolmycin (**9**). In inhibition assays of in vitro growth of *Plasmodium falciparum*, compounds **1**–**6** exhibited antiplasmodial activity against drug-sensitive line 3D7 and drug-resistant line Dd2 of *P. falciparum*. In this paper, we report the

Received: May 11, 2011

dx.doi.org/10.1021/np200399t | *J. Nat. Prod.* XXXX, XXX, XXX–XXX

Continuous transcutaneous submental electrical stimulation in obstructive sleep apnea: a feasibility study

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Word count: 2499 (manuscript) / 203 (abstract)

Keywords: Electromyogram, respiratory muscles, diaphragm

Short title: Genioglossus stimulation in OSA

How does this advance the field This study helps to define the value of continuous transcutaneous electrical stimulation of the genioglossus muscle in patients with sleep apnea. ***What are the clinical implications*** In normal awake subjects transcutaneous electrical stimulation causes contraction of the tongue muscles. In patients with obstructive sleep apnea snoring and sleep-disordered breathing can be reduced by continuous stimulation if low current is used to avoid arousal from sleep.

Australasian Journal of Dermatology

Australasian Journal of Dermatology (2011) **52**, e15–e18 doi: 10.1111/j.1440-0960.2010.00658.x

ORIGINAL ARTICLE

Detection of $CD4^+CD25^+FOXP3^+$ regulatory T cells in peripheral blood of patients with chronic autoimmune urticaria

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ABSTRACT

Background/Objectives: Compelling evidence indicates a significant role for a population of $CD4^+$ T regulatory cells in suppressing immune responses and in maintaining immunological homeostasis. This study aims to investigate the potential role of $CD4^+CD25^{HIGH}FOXP3^+$ T regulatory cells in patients with chronic autoimmune urticaria and to define the characteristics of $CD4^+CD25^{HIGH}FOXP3^+$ cells in chronic urticaria.

Methods: We used flow cytometry to assess the expression of $CD4^+CD25^{HIGH}FOXP3^+$ cells in the peripheral blood mononuclear cells of patients with chronic autoimmune urticaria.

Results: In this study, we found that patients with chronic autoimmune urticaria have a significantly reduced frequency of $CD4^+CD25^{HIGH}FOXP3^+$ cells ($1.39 \pm 0.27\%$ *vs* $2.09 \pm 0.34\%$; $P = 0.001$) in their peripheral blood, accompanied by a decreased intensity of FOXP3 expression (50.13 ± 9.79 *vs* 68.19 ± 6.40; $P < 0.001$). Notably, although patients with chronic idiopathic urticaria had a reduced frequency of $CD4^+CD25^{HIGH}FOXP3^+$ cells ($1.85 \pm 0.46\%$ *vs* $3.64 \pm 0.48\%$; $P < 0.001$), their FOXP3 expression levels did not differ from those in healthy controls.

Conclusions: Patients with chronic autoimmune urticaria displayed a reduced percentage of $CD4^+CD25^+FOXP3^+$ regulatory T cells. The results imply $CD4^+CD25^+FOXP3^+$ regulatory T cells may contribute to the autoimmune pathological process of chronic autoimmune urticaria.

Key words: chronic autoimmune urticaria, FOXP3, regulatory T cell.

INTRODUCTION

In 80–90% of patients with chronic urticaria (CU), no specific underlying cause is found, although there is a subset of these patients in whom autoantibodies to the high-affinity immunoglobulin E receptor FcεRI are found.[1] This subgroup is labelled chronic autoimmune urticaria (CAU).[2] It was later demonstrated that these antibodies stimulated the release of histamine. Antithyroid antibodies are reported to be more frequently found in patients with autoimmune urticaria.[3]

Examination of biopsies from urticaria patients demonstrated that most of the infiltrating T cells possessed a

Abbreviations:

CAU	chronic autoimmune urticaria
CIU	chronic idiopathic urticaria
CU	chronic urticaria
FOXP3	forkhead box P3
IgE	immunoglobulin E
mAb	monoclonal antibodies
MFI	mean fluorescence intensity
PBMC	peripheral blood mononuclear cells
Th	T helper
Treg	T regulatory

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Submitted 30 November 2009; accepted 7 April 2010.

International Journal of Antimicrobial Agents 37 (2011) 152–155

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Short communication

High prevalence of plasmid-mediated quinolone resistance determinant *aac(6′)-Ib-cr* amongst *Salmonella enterica* serotype Typhimurium isolates from hospitalised paediatric patients with diarrhoea in China

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ARTICLE INFO

Article history:
Received 16 April 2010
Accepted 18 October 2010

Keywords:
qnr
aac(6′)-Ib-cr
Salmonella enterica serotype Typhimurium

ABSTRACT

In this study, the antimicrobial susceptibilities and prevalence of plasmid-mediated quinolone resistance determinants amongst *Salmonella enterica* serotype Typhimurium isolates from hospitalised paediatric patients with diarrhoea in China were investigated. In total, 40 (64.5%) of 62 *S.* Typhimurium isolates were resistant to ciprofloxacin (minimum inhibitory concentration ≥0.5 μg/mL), comprising 28 isolates with low-level resistance and 12 isolates with high-level resistance. All ciprofloxacin-resistant isolates were multiresistant to other antimicrobial agents. Four pulsed-field gel electrophoresis (PFGE) clusters were found amongst the 40 ciprofloxacin-resistant isolates, amongst which PFGE clusters A, B, E and D accounted for 7, 4, 1 and 28 isolates, respectively. Two isolates with high-level ciprofloxacin resistance had two mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC*. The remaining ciprofloxacin-resistant isolates had only one mutation in the QRDR of *gyrA*. All 62 *S.* Typhimurium isolates were negative for *qnr* genes and *qepA* and 23 (37.1%) of the isolates were positive for *aac(6′)-Ib-cr*. Nineteen isolates harbouring *aac(6′)-Ib-cr* belonged to PFGE cluster D. A high prevalence of ciprofloxacin resistance and *aac(6′)-Ib-cr* was found amongst *S.* Typhimurium isolates in China from hospitalised paediatric patients with diarrhoea not receiving quinolones. A single mutation in the QRDR of *gyrA* as well as production of AAC(6′)-Ib-cr contributed to ciprofloxacin resistance. Clonal spread was responsible for the dissemination of *aac(6′)-Ib-cr* amongst *S.* Typhimurium isolates.

1. Introduction

Non-typhoidal *Salmonella* spp., particularly *Salmonella enterica* serotype Typhimurium, are one of the most common bacterial pathogens causing enteric infections amongst paediatric patients [1]. Antimicrobial agents are not essential for the treatment of most *Salmonella* infections. However, treatment of severe infections caused by *Salmonella* spp. is necessary [2]. Broad-spectrum cephalosporins are commonly used to treat invasive infections or severe diarrhoea [3]. In recent years, however, cephalosporin-resistant *Salmonella* isolates in humans, particularly *S.* Typhimurium isolates, have frequently been reported and have become increasingly common worldwide [4]. Although fluoroquinolones (FQs) are not recommended as a choice of treatment for infections in paediatric patients because of their potential to cause arthropathy, FQs are still one of the last treatment options for life-threatening *Salmonella* infections caused by multidrug-resistant isolates [5]. However, FQ resistance amongst these isolates has become increasingly common worldwide [6]. In China, a high prevalence of FQ resistance was detected amongst *Salmonella* isolates from infections in humans, especially amongst *S.* Typhimurium isolates [5]. Quinolone resistance mainly results from chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC and ParE).

Plasmid-mediated quinolone resistance (PMQR) mechanisms, including QepA efflux, QNR proteins and AAC(6′)-Ib-cr, were found to confer decreased susceptibility to FQs [7]. The aim of this study was to investigate mutations in the QRDRs of *gyrA*, *gyrB*, *parC* and

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0924-8579/$ – see front matter © 2010 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
doi:10.1016/j.ijantimicag.2010.10.021

Journal of Clinical Virology 52 (2011) 107–112

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Identification and characterization of acyclovir-resistant clinical HSV-1 isolates from children

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ARTICLE INFO

Article history:
Received 29 March 2011
Received in revised form 17 June 2011
Accepted 24 June 2011

Keywords:
HSV-1
Acyclovir
Resistance
Children
Assay
Clinical isolates

ABSTRACT

Background: The occurrence of herpes simplex virus (HSV) with acyclovir (ACV) resistance is a cause for concern due to the frequent use of ACV for treatment, suppressive therapy, and prophylaxis of HSV infection. Although HSV infection is prevalent among children, very little is known about the drug susceptibility of HSV circulating in this patient population.
Objective: To determine the status of ACV resistant HSV-1 among children.
Study design: A reporter cell-based HSV infection assay (mVILA) was developed to conveniently evaluate the ACV susceptibility of HSV-1 clinical strains and used to analyze 68 HSV-1 primary isolates from oral lesions in children.
Results: Compared with PRA, mVILA is easier to perform. Using mVILA, HSV-1 isolates C106, C153, and C174 were found completely resistant to ACV, with a greater than 100-fold increase in IC50s. Sequence analysis of thymidine kinase (TK) and DNA polymerase (DNA POL) genes identified 11 new mutations. Structural modeling of the TK and DNA POL proteins suggested structural changes that might alter their interactions with ACV and ACV triphosphate, respectively. The insertion of a single G in a seven-guanine homopolymeric repeat sequence generated a truncated TK protein in C106.
Conclusion: This study provides preliminary data on the ACV susceptibility status of HSV-1 in children. The prevalence rate of ACV-resistant HSV-1 in children was higher than predicted. Moreover, multiple mechanisms leading to the resistance were identified. These results suggest that new anti-herpetics with different working mechanisms should be valuable.

1. Background

Acyclovir (ACV) is the first selective antiviral drug to be characterized; it has been the drug of choice for the prophylaxis and treatment of primary and recurrent HSV infection for more than two decades now. ACV is a nucleoside analog prodrug that can be converted into an ACV monophosphate by the HSV thymidine kinase (TK) within HSV-infected cells. Cellular kinases subsequently add two more phosphates to form the active drug ACV triphosphate (ACV-TP). The ACV-TP competes with 2-deoxyguanosine triphosphate as a substrate for viral DNA polymerase (DNA POL), thus terminating DNA synthesis upon its incorporation. Over the years, ACV-resistant (ACV-r) HSV mutant strains have been isolated from both immunocompromised and immunocompetent adult patients.[1,2] Using standardized or non-standardized methods, the prevalence rates of ACV-r HSV were reported vary from 3.5% to 10.9% in immunocompromised patients and from 0.3% to 0.7% in immunocompetent patients.[2–9] Among these ACV-r HSV mutant strains, 95% and 5% are caused by TK gene mutations and the viral DNA pol gene, respectively.[7,10]

Studies on ACV-r HSV strains have suggested that TK mutants produce distinctly altered TK enzymes, such as TK-negative mutants, mainly because of the disruptive characteristic of the TK ORF through deletion/insertion mutants, and TK mutants with reduced ACV sensitivity from the structural alteration of the TK protein.[11] Although rare, HSV DNA POL mutants have been identified from ACV-r clinical isolates.[12,13]

Children are susceptible to infection by HSV-1, a common cause of ulcers in the mouth, gingivostomatitis, cold sores, or oral/labial lesions. However, the status of ACV-r HSV among children has not

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doi:10.1016/j.jcv.2011.06.009

Improving Transfection of Human Pulmonary Epithelial Cells by Doping LMW-PEI-*g*-Chitosan with β-Estradiol

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Received 22 June 2010; accepted 10 October 2010
DOI 10.1002/app.33584
Published online 25 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: PEI-grafted chitosan (PEI-CMCS) copolymer was synthesized through EDC-mediated amidation reaction between carboxymethyl chitosan and low-molecular-weight polyethyleneimine (LMW PEI). PEI-CMCS and CS/DNA complexes were characterized. Compared with pristine chitosan, the PEI-CMCS exhibited an enhanced ability to condense DNA. Incorporation of LMW PEI to chitosan was found to achieve higher transfection efficiency and much lower cytotoxicity than PEI25K at an optimum weight ratio of 15 in COS-7 cells. However, PEI-CMCS was incompetent to transfer refractory pulmonary epithelial cells. It was demonstrated that doping complex medium or treating cells with a steroid hormone—β-estradiol significantly improved the transfection efficiency of 16HBE and A549 cells. This study suggests that steroid hormone may become an additive for other cationic polymers to facilitate gene transfection in pulmonary cells. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 874–882, 2011

Key words: chitosan; polyethyleneimine; gene delivery; pulmonary epithelial cell; β-estradiol

INTRODUCTION

The lung is an attractive target for gene therapy since it is easily accessible and represents the organ where lethal congenital and acquired diseases occur.[1] Clinical pulmonary disorders, such as cystic fibrosis (CF), are suitable candidates for gene therapy.[2] The growing interest in nonviral vector systems has spurred researchers to develop advanced materials for delivery of DNA and siRNA to lung cells with high efficiency and low toxicity.

Of the materials utilized as nonviral vectors, polyethyleneimine (PEI), a synthetic polycation, is the most widely investigated because of its strong ability to condense DNA and transfect a broad range of cell lines with high efficiency.[3] The transfection efficiency of PEI is closely related to its structure and molecular weight. Generally, the PEI with high molecular weight shows high efficiency, but has high cytotoxicity.[4,5] Although low-molecular-weight PEIs have lower cytotoxicity, their efficiency deteriorates with the drop of molecular weight.

Chitosan, comprised of β-(1-4) linked 2-amino-2-deoxy-D-glucose, has been proposed as one of the potential nonviral vectors for gene transfer, benefiting from its cationic character, biodegradability, and biocompatibility.[6] However, the clinical use of chitosan is limited by the low-transfection efficiency.[7] To improve transfection efficiency of chitosan polyplexes, numerous modifications to the polymer structure have been made. Several strategies were utilized to improve the cationic property of chitosan.[8–10] To increase the buffering capacity of chitosan, two important modifications were investigated. One attempt was to conjugate chitosan with varying ratios of urocanic acid. These imidazole-containing derivatives showed reduced cytotoxicity and significantly enhanced transfection efficiency due to the increased buffering capability.[11] The same buffering capacity was also achieved by conjugating chitosan with polyethyleneimine. In particular, grafting low-molecular-weight PEI to chitosan could achieve comparable or even superior transfection efficiency to PEI25K, but exhibited much lower cytotoxicity.[12–14] Nonetheless, to the best of our knowledge, no work has been reported on exploring PEI-chitosan as a gene delivery system for refractory human pulmonary epithelial cells thus far.

In this work, aiming at designing a low cytotoxic nonviral vector for airway gene delivery, we synthesized PEI-grafted chitosan (PEI-CMCS) copolymer

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Contract grant sponsor: National 863 High-Tech Project, People's Republic of China; contract grant number: 2007AA022002.

Cancer Letters 313 (2011) 54–63

Contents lists available at SciVerse ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Inactivation of Rac1 reduces Trastuzumab resistance in PTEN deficient and insulin-like growth factor I receptor overexpressing human breast cancer SKBR3 cells

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ARTICLE INFO

Article history:
Received 17 June 2011
Received in revised form 21 August 2011
Accepted 22 August 2011

Keywords:
Trastuzumab resistance
Rac1
PTEN
IGF-IR
Akt
HER2

ABSTRACT

Drug resistance remains to be a big challenge in applying anti-HER2 monoclonal antibody Trastuzumab for treating breast cancer with HER2 overexpression. Amplification of insulin-like growth factor I receptor (IGF-IR) and deletion of tumor suppressor phosphatase and tensin homolog (PTEN) are implicated in Trastuzumab resistance, however, the underlying mechanisms have not been clearly defined. Activation of Rac1, a member of Rho GTPase family, is capable of causing cytoskeleton reorganization, regulating gene expression and promoting cell proliferation. To investigate the mechanism of Trastuzumab resistance, PTEN knockdown and IGF-IR overexpressing stable cell lines were generated in HER2 overexpression human breast cancer SKBR3 cells. Rac1 was highly activated in PTEN deficient and IGF-IR overexpressing Trastuzumab-resistant cells in a HER2-independent manner. Inactivation of Rac1 by using a Rac1 inhibitor NSC23766 or siRNA knocking down the expression of Tiam1, a guanine nucleotide exchange factor for Rac, significantly reduced Trastuzumab resistance in SKBR3 cells. Inhibition of Rac1 had no effect on the levels of phosphor-HER2 and phosphor-Akt, but significantly decreased the levels of cyclin D1 in Trastuzumab-resistant cells. Inhibition of Akt with an Akt inhibitor also significantly reduced Trastuzumab resistance. However, simultaneous inhibition of both Rac1 and Akt resulted in a significantly more decrease of Trastuzumab resistance than inactivation of Rac1 or Akt alone. These results suggest that Rac1 activation is critically involved in Trastuzumab resistance caused by PTEN deletion or IGF-IR overexpression. Simultaneous inhibition of Rac1 and Akt may represent a promising strategy in reducing Trastuzumab resistance in HER2 overexpression breast cancer.

1. Introduction

Human epidermal growth factor receptor-2 (HER2 or ERBB2) is overexpressed in approximately 15–25% of human breast cancers and HER2 overexpression is associated with poor prognosis [1,2]. Trastuzumab (Herceptin) is the first rationally designed anti-HER2 monoclonal antibody approved for the treatment of breast cancers with HER2 overexpression. While wide applications of Trastuzumab achieved clinical therapeutic efficacy in some patients with HER2 overexpression, a large portion of selected patients did not respond well to Trastuzumab treatment. It was reported that the overall response rate to Trastuzumab treatment as a single agent was 23–26%

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doi:10.1016/j.canlet.2011.08.023

Protein expression status of p53 and epidermal growth factor receptor in thymoma

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Received September 9, 2010; Accepted December 27, 2010

DOI: 110.3892/ol.2011.267

Abstract. Evaluating the state of malignancy of thymoma is a challenge due to the discrepancies between pathological classifications and clinical stage criteria. Therefore, the identification of markers contributing to the assessment of the stage of malignancy of thymoma would be useful. This study aimed to evaluate the prevalence of p53 and epidermal growth factor receptor (EGFR) in thymoma, demonstrate the clinical significance and assess the potential of specific-targeted therapy. A total of 16 thymic hyperplasia patients and 43 thymoma patients were included in the study. Samples were tested for the expression of p53 and EGFR using immunohistochemistry. In the multivariate analysis, the thymoma cases were followed up to analyze the relationship between survival rates and a number of potential factors, such as p53 and EGFR. There were 21 completely resected stage II cases which were evaluated for relapse-free time. The distribution of p53 with clinicopathological parameters was: type A/AB 57.1%, type B1 85.7%, type B2 85.7%, type B3 90.9% and type C 100%; and stage I 90.9%, stage II 90.9%, stage III 100% and stage IV 100%. The EGFR-positive rates were: type A/AB 42.9%, type B1 71.4%, type B2 57.1%, type B3 90.9% and type C 100%; and stage I 45.5%, stage II 76%, stage III 75% and stage IV 100%. EGFR expression correlated with tumor size, pathological classification and advanced clinical stage, whereas p53 correlated only with pathological classifications. Findings of our multivariate analysis showed that neither p53 nor EGFR are independent prognostic factors. Nevertheless, the fact that a statistical difference ($p<0.05$) was noted in relapse-free survival time between the EGFR-positive and EGFR-negative groups (48.182±33.757 vs. 76.3±10.339 months) suggests that EGFR plays a key role in thymoma progression. No positive results were found between the p53 groups following a survey conducted to assess relapse time. Therefore, the application of EGFR-targeting agents is warranted in invasive thymoma, whereas the targeting of p53 has yet to be elucidated.

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Key words: p53, epidermal growth factor receptor, thymoma, clinicopathological parameters, survival

Introduction

Thymoma is a rare anterior mediastinal tumor with complicated processing mechanisms. The pathological classifications were based on the WHO classification system. Thymomas were classified into types A, B and C (1). On the other hand, the current clinical diagnosis is predominantly based on the estimate of surgical management established by Masaoka *et al* (2). However, pathologically indolent cases occasionally show malignancy as invasion, recurrence or metastasis (3). For this reason, discrepancies are noted between clinical stages and the current pathological classifications. Therefore, certain markers should be defined as a complement of clinicopathological parameters to evaluate malignancy and outcome. Surgical resection is the optimal standard therapy. Based on the large-sample analysis of 1,320 cases in Japan, surgical treatment was shown to be an independent factor affecting prognosis, but the value of adjuvant chemotherapy and radiotherapy is uncertain (4). However, in terms of the unresectable or relapsed cases, a non-surgical therapeutic approach is the last recourse. Thus, alternative therapeutic molecular targets are required.

As a key indicator, mutant p53 is predisposed to cancer (5). Certain authors studied the accumulation of an abnormal form of the protein in the cell nucleus, the normal p53 protein with a half-life that was too short to be detected, while most, but not all, mutant p53 proteins possess a prolonged half-life, accumulate in tissues, and are able to be directly detected by immunohistochemical assays (6,7). To exclude false-positive and false-negative results, the analysis was performed a number of times according to the most frequently used method, i.e., immunohistochemistry with certain specific antibodies, such as DO-7. p53 protein accumulation is interpreted as being indicative of the presence of p53 mutations. As such, the immunohistochemical evaluation of p53 revealed its prognostic significance in the tumor (8,9).

Epidermal growth factor receptor (EGFR), a cellular surface membrane glycoprotein receptor, plays a key role in the regulation of key normal cellular processes and in the hyperproliferation, infiltration, invasion and metastasis of

ARTICLE
Journal of Cellular Biochemistry 112:3313–3321 (2011)

Journal of Cellular Biochemistry

Nobiletin, a Polymethoxylated Flavonoid from Citrus, Shows Anti-Angiogenic Activity in a Zebrafish In Vivo Model and HUVEC In Vitro Model

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ABSTRACT

Traditional Chinese medicinal herbs are a rich source of compounds with reported anti-inflammatory and anti-carcinogenic effects. Growing evidence shows the codependence of chronic inflammation and angiogenesis, and the potential benefits of targeting angiogenesis in the treatment of chronic inflammation and targeting inflammation in the treatment of diseases with impaired angiogenesis. We hypothesized that the anti-inflammatory activity of the natural compounds may owe at least some of its efficacy to their anti-angiogenic activity and hence we investigated the anti-angiogenic activity of these compounds in vivo in zebrafish embryos and in vitro in human umbilical vein endothelial cells (HUVECs). Nobiletin, a polymethoxylated flavonoid from citrus fruits, showed anti-angiogenic activity in both assays. Nobiletin inhibited the formation of intersegmental vessels (ISVs) in live transgenic zebrafish embryos expressing green fluorescent protein (GFP) in the vasculature. Cell cycle analysis of dissociated zebrafish embryo cells showed that nobiletin induced G0/G1 phase accumulation in a dose-dependent manner in GFP-positive endothelial cells. Nobiletin also dose-dependently induced *VEGF-A* mRNA expression. In HUVECs, nobiletin inhibited endothelial cell proliferation and, to a greater extent, tube formation in a dose-dependent manner. As in the in vivo study, nobiletin induced G0/G1 cell cycle arrest in HUVECs. However, this arrest was not accompanied by an increase in apoptosis, indicating a cytostatic effect of nobiletin. This study, for the first time, identifies nobiletin as having potent anti-angiogenic activity and suggests that nobiletin has a great potential for future research and development as a cytostatic anti-proliferative agent. J. Cell. Biochem. 112: 3313–3321, 2011.

KEY WORDS: NOBILETIN; FLAVONOID; ANTI-INFLAMMATORY; ANGIOGENESIS; ZEBRAFISH; CELL CYCLE

Angiogenesis plays an important role in the development of cancer and chronic inflammatory diseases including psoriasis, retinopathy, and rheumatoid arthritis [Folkman, 1995; Jackson et al., 1997]. There is growing evidence to suggest that chronic inflammation and angiogenesis are codependent, involving increased cellular infiltration and proliferation as well as over-lapping roles of regulatory growth factors and cytokines [Jackson et al., 1997]. Pharmacology of many anti-inflammatory drugs revealed at least some part of their efficacy was due to their anti-angiogenic effect [Jackson et al., 1997].

Traditional Chinese medicinal herbs have been used for thousands of years for treatment of chronic inflammation and

Kai Heng Lam and Deepa Alex contributed equally to the work.

Additional supplementary information may be found in the online version of this article.

Grant sponsor: Science and Technology Development Fund of Macau SAR; Grant sponsor: Research Committee, University of Macau.

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Received 29 November 2010; Accepted 29 June 2011 • DOI 10.1002/jcb.23257 • © 2011 Wiley Periodicals, Inc.
Published online 11 July 2011 in Wiley Online Library (wileyonlinelibrary.com).

Acta Oto-Laryngologica, 2011; 131: 533–538

informa
healthcare

ORIGINAL ARTICLE

The specific IgE reactivity pattern of weed pollen-induced allergic rhinitis patients

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Abstract

Conclusion: Specific immunoglobulin E (IgE) reactivity towards the major mugwort allergen Art v 1 is a good indicator for Art v sensitization. Allergens from the ragweed species Amb t and Amb a possibly share common IgE-binding epitopes. *Objective:* The aim of this study was to investigate the reactivity pattern of IgE in Chinese patients with weed pollen-induced allergic rhinitis. *Methods:* Sera from 50 weed pollen-induced allergic rhinitis patients were tested for specific serum IgE reactivity against allergenic extracts of mugwort (Artemisia vulgaris, Art v), short ragweed (Ambrosia artemisiifolia, Amb a), giant ragweed (Ambrosia trifida, Amb t), and single allergens of Art v 1, Art v 3, Amb a 1, and profilin. *Results:* Sera from 88% of the patients demonstrated positive specific IgE reactivity to Art v, and of these 82% were positive to Art v 1. Sera from 38% of the patients showed positive specific IgE reactivity to both ragweed species Amb t and Amb a. A strong correlation was found between the specific IgE levels of Amb t and Amb a. Of the Amb a IgE-positive patients, 38% were positive for Amb a 1. Of all patient sera tested, 12% were specific IgE-positive to profilin.

Keywords: *Mugwort, ragweed, major allergen, profiling*

Introduction

Mugwort (Artemisia vulgaris, Art v) and ragweed (Ambrosia artemiisifolia, Amb a, and Ambrosia trifida, Amb t) are allergenic weeds belonging to the family Asteraceae (Compositae). Both mugwort and ragweed are widespread in northern parts of China and their pollens are considered to be responsible for eliciting immunoglobulin E (IgE)-mediated allergic symptoms during late summer and autumn [1,2]. A national allergen prevalence study performed by the China Alliance of Research on Respiratory Allergic Disease (CARRAD) group showed that the prevalence of positive skin prick test (SPT) reactions among allergic subjects in northern China was 27% for mugwort and 18% for ragweed [3]. This prevalence is in agreement with that of 27% of patients with nasal symptoms visiting the Allergy Clinic of Beijing Institute of Otolaryngology that were found to be SPT-positive to mugwort and 13% of patients that were SPT-positive to ragweed [2]. It indicates that the ragweed/mugwort sensitization problem is global, as it exists in China in addition to Europe and the USA. Most of the weed-allergic patients visiting the otolaryngology clinic had SPT-positive reactions to both mugwort and ragweed. While patients that are exclusively SPT-positive to ragweed are rare, SPT reactions to mugwort alone are more common. Based on pollen counts [4,5], the flowering periods of mugwort and ragweed are almost completely overlapping, making it difficult to distinguish between ragweed or mugwort pollen-induced allergic reactions.

In China, specific diagnosis and identification of the primary sensitizing weed species is hampered because many allergy clinics do not have access to

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(Received 10 September 2010; accepted 1 November 2010)

ISSN 0001-6489 print/ISSN 1651-2251 online © 2011 Informa Healthcare
DOI: 10.3109/00016489.2010.539265

Respiratory Medicine (2011) 105, 1543–1549

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Weight of the IDSA/ATS minor criteria for severe community-acquired pneumonia☆

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Received 16 March 2011; accepted 21 June 2011
Available online 20 July 2011

KEYWORDS
Community-acquired pneumonia;
Severity;
Minor criteria;
Association;
Weight

Summary
Background: The 2007 Infectious Disease Society of America (IDSA)/American Thoracic Society (ATS) guidelines defined severe community-acquired pneumonia (CAP) when patients fulfilled three out of nine minor criteria. Whether each of the criteria is of equal weight is not clear. The purpose of this study was to determine the weight of the minor criteria.
Methods: 1230 adult patients admitted to our hospital from 2005 to 2009 for CAP were reviewed retrospectively.
Results: Hospital mortality rose sharply from 0.3%, 1.0% and 3.3%, respectively, for patients with none, one and two minor criteria to 10.5% for patients with three minor criteria. Arterial oxygen pressure/fraction inspired oxygen (PaO_2/FiO_2) $\leq$ 250 mm Hg, confusion, and uremia had the strongest association with mortality (Odds ratio, 22.162, 22.148, 16.343; respectively). Leukopenia, hypothermia, and hypotension were not associated with mortality. Confusion and uremia showed independent relationships with mortality (Odds ratio, 9.296, 8.493; respectively). Sequential organ failure assessment (SOFA) scores and costs increased significantly with the number of minor criteria present. Uremia and $PaO_2/FiO_2 \leq 250$ mm Hg were most

☆ The abstract (NO. 503) has been accepted for Oral Presentation at the ERS (European Respiratory Society) Amsterdam 2011 Annual Congress (24-28 September).

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doi:10.1016/j.rmed.2011.06.010