中山大学热带病防治研究教育部重点实验室

Key Laboratory of Tropical Disease Control (Sun Yat-Sen University), Ministry of Education

2016 年学术年报

中山大学中山医学院

广　州

2017 年 9 月

中山大学热带病防治研究教育部重点实验室

实验室负责人

重点实验室主　任：吴忠道

重点实验室副主任：余新炳、吴长有、江丽芳、陈省平

重点实验室秘　书：关苑君

Key Laboratory of Tropical Disease Control (Sun Yat-Sen University)

Ministry of Education

Director of Key Laboratory:	Wu Zhongdao
Deputy Director of Key Laboratory:	Yu Xinbing, Jiang Lifang, Wu Changyou, Chen Shengping
Secretary of Key Laboratory:	Guan Yuanjun

中山大学热带病防治研究教育部重点实验室
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黎孟枫　教授　　（中山大学中山医学院）

张　辉　教授　　（中山大学中山医学院）

前　言

中山大学热带病防治研究教育部重点实验室于 2003 年批准立项建设（教技函[2003]57 号），于 2007 年通过验收并正式开放运行（教技函[2007]55 号），2010 年评估获得“优秀”。重点实验室发展定位为：针对在热带亚热带流行、以及在这些地区起源并流行至其他区域的感染性疾病，在开展病原致病机制及其分子遗传学、宿主免疫及其病理损伤机制等先导性研究的基础上，紧扣战略发展重点，突破分子诊断、特异性药物和防控策略中的关键理论和技术。本重点实验室围绕与热带亚热带气候环境相关疾病，结合国际前沿研究方向，经过几年建设，已构建了病原基因组学、病原蛋白质组学、贵重仪器共享以及生物安全实验设施等平台，并在重要热带病特异性药物研发与免疫细胞的再生工程，热带亚热带流行病病原的分子诊断及防控策略等方向形成了研究特色和优势，并取得重要的进展和成果。

实验室 2016 年固定人员 69 人，其中教授 45 人，副教授 16 人，拥有博士学位者 59 人，硕士学位者 9 人。通过学校“211 工程”、“985 工程”等建设支持，本重点实验室条件得到了全面改善。实验室面积达 9200 平方米，仪器设备总值 5100 多万元，其中 10 万元以上设备 80 台（套）。已初步建成了具有国际水平的热带病防治研究中心，大大增强了我国在热带病防治研究上的国际竞争力。2016 年新增纵向科研项目 44 项，项目总经费达 3811.49 万元。2016 年实验室发表论文 131 篇，其中 SCI 收录论文 107 篇，申请国家专利 10 项，获得授权专利 19 项，获得国家医疗器械证书 14 项。人才培养方面，培养博士研究生 41 名，硕士研究生 52 名。

本集年报收录了实验室 2016 年发表的部分论文摘要，以期反映实验室的研究现状、研究进展和科研成果。

中山大学中山医学院
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实验室主任：吴忠道
2017 年 9 月

目　录

2016 年实验室固定人员

序号	姓名	性别	学位	职称	专业
1	詹希美	男	博士	教授	人体寄生虫学
2	江丽芳	女	硕士	教授	病原生物学
3	余新炳	男	博士	教授	人体寄生虫学
4	何蕴韶	男	博士	教授	病原诊断技术
5	凌文华	男	博士	教授	流行病学
6	蒋玮莹	女	博士	教授	遗传学
7	吴长有	男	博士	教授	免疫学
8	袁岩	男	博士	教授	病毒学
9	席丽艳	女	博士	教授	皮肤病与性病学（真菌）
10	古洁若	女	硕士	教授	免疫学
11	张辉	男	博士	教授	病毒学
12	Dmitry Isaak Gabrilovich	男	博士	教授	免疫学
13	吕芳丽	女	博士	教授	人体寄生虫学学
14	刘焕亮	男	博士	教授	病毒学
15	吴忠道	男	博士	教授	寄生虫学学
16	曹开源	男	博士	教授	免疫学
17	高志良	男	博士	教授	免疫学
18	黄曦	男	博士	教授	免疫学
19	赖小敏	男	硕士	教授	免疫学
20	黎孟枫	男	博士	教授	病原生物学
21	李刚	男	博士	教授	病原诊断技术
22	陆家海	男	博士	教授	流行病学
23	郑小英	女	硕士	副教授	虫媒学
24	潘景轩	男	博士	教授	病原生物学

25	高国全	男	博士	教授	分子生物学
26	张晋昕	男	博士	副教授	分子生物学
27	胡旭初	男	博士	副教授	寄生虫学
28	郝元涛	男	博士	教授	流行病学
29	周家国	男	博士	教授	病原生物学
30	陶海燕	女	博士	副教授	地理模拟与疾病监控
31	周兴旺	男	博士	教授	分子寄生虫学
32	李学荣	男	博士	教授	医学微生物学
33	洪海	女	博士	副教授	免疫学
34	李隽	男	博士	教授	病原生物学
35	郭学敏	女	博士	教授	病原生物学
36	顾怀宇	男	博士	教授	微生物学
37	周毅	男	博士	副教授	免疫学
38	周洁	女	博士	教授	免疫学
39	吕志跃	男	博士	教授	寄生虫学
40	李义平	男	博士	教授	分子病毒学
41	王琴	女	博士	教授	医学微生物学
42	蔡卫斌	男	博士	教授	人体寄生虫学
43	罗海华	女	硕士	实验师	病毒学
44	吴瑜	女	硕士	副教授	分子生物学
45	徐霖	女	博士	副教授	微生物学
46	张萍	女	博士	教授	病原生物学
47	夏敏	男	博士	教授	统计学
48	柏川	男	博士	副教授	免疫学
49	罗海彬	男	博士	教授	流行病学
50	黄艳	女	博士	副教授	寄生虫学
51	付清玲	女	博士	教授	免疫学
52	袁洁	女	博士	副教授	病原生物学
53	刘超	男	博士	副教授	病毒学

54	曾谷城	男	博士	教授	免疫学
55	朱勋	男	博士	副教授	分子生物学
56	田国宝	男	博士	副教授	免疫学
57	李博	男	博士	教授	分子病毒学
58	孙希	女	博士	副教授	寄生虫学
59	杨克礼	男	博士	教授	免疫学
60	陈小舒	女	博士	教授	免疫学
61	吴敏昊	女	博士	教授	免疫学
62	蔡俊超	男	博士	副教授	病原生物学
63	潘婷	女	博士	特聘研究员	病毒学
64	袁广卿	女	中专	高级实验师	生物安全
65	胡黎平	男	博士	高级实验师	设备管理
66	吴珏珩	女	硕士	高级实验师	设备管理
67	李美玉	女	硕士	实验师	设备管理
68	陈省平	男	博士	副研究员	实验室管理
69	关苑君	女	硕士	助理实验师	实验室管理

2016 年实验室新增科研项目和获得科研经费

项目名称	项目编号	负责人	项目分类	项目子类	立项日期	计划完成日期	合同经费
非编码 RNA 在 HIV-1 潜伏感染的建立和维持过程中分子机理研究	81561128007	张辉	国家自然科学基金	国际(地区)合作与交流项目/NSFC-RGC 联合资助项目	2016/1/1	2018/12/31	299.96
NF-κB 信号通路反馈性泛素化网络失控促食管非可控炎症诱导恶性转 化的分子机制	91529301	李隽	国家自然科学基金	重大研究计划/重点支持项目	2016/1/1	2018/12/31	297.6
炎性微环境调控恶性肿瘤发生发展的机制研究	81621004	黎孟枫	国家自然科学基金	参与项目	2016/9/1	2022/12/31	162.5
炎性微环境调控恶性肿瘤发生发展的机制研究	81621004	李隽	国家自然科学基金	参与项目	2016/9/1	2022/12/31	162.5
长非编码 RNA CXorf28 促进非小细胞肺癌转移的分子机制	81672296	吴珏珩	国家自然科学基金	面上项目	2016/9/1	2020/12/31	60
结核及耐药结核感染中 CD244 的免疫功能与机制	31670879	曾谷城	国家自然科学基金	面上项目	2016/9/1	2020/12/31	60
人结核抗原特异性 CD4+ T 细胞的体内免疫防御功能及机制研究	31670900	吴长有	国家自然科学基金	面上项目	2016/9/1	2020/12/31	60
NLRP6 介导的角膜保护作用及分子调控机制	31670880	吴敏昊	国家自然科学基金	面上项目	2016/9/1	2020/12/31	60
IFI16 在卡波氏肉瘤相关疱疹病毒感染和致病中的作用和机制	81671996	况二胜	国家自然科学基金	面上项目	2016/9/1	2020/12/31	57
PSM-E 在前列腺癌中诱导肿瘤相关巨噬细胞凋亡的功能及机制研究	81672556	曹开源	国家自然科学基金	面上项目	2016/9/1	2020/12/31	52
2015 海峡两岸热带医学研究学术交流研讨会	81581260456	黄曦	国家自然科学基金	国际(地区)合作与交流项目	2016/8/19	2015/12/31	1.5
重要热带病传播相关的入侵媒介生物及其病原体的生物学特性研究（外拨 3 个课题）	2016YFC1200500	吴忠道	科技部	国家重点研发计划项目	2016/7/1	2018/12/31	893.9
入侵媒介生物的入侵致害相关生物学特性研究	2016YFC1200501	吴忠道	科技部	国家重点研发计划课题	2016/7/1	2018/12/31	383.1

病原传播相关入侵生物分子标识与精准溯源技术研究	2016YFC1202003	吕志跃	科技部	国家重点研发计划课题	2016/7/26	2018/12/31	215
入侵媒介生物数据共享平台建设	2016YFC1202005	吕志跃	科技部	国家重点研发计划参与	2016/7/26	2018/12/31	50
入侵媒介生物的生态适应性及进化研究	2016YFC1200502	何蔼	科技部	国家重点研发计划参与	2016/7/1	2018/12/31	48.11
入侵生物的种质遗传学及适应性分子基础研究	2016YFC1200503	孙希	科技部	国家重点研发计划参与	2016/7/1	2018/12/31	46.82
寄生虫种质资源保藏		吕志跃	科技部	其他	2016/10/7	2020/12/31	15
TNFRSF18 在抗结核免疫中的作用及机制研究	2016A030306004	吴敏昊	广东省自然科学基金	杰出青年基金项目	2016/6/1	2020/6/1	100
HIV-1 通过 let-7i 微小 RNA 调控白介素-2 表达的机制研究	2016A030313826	罗海华	广东省自然科学基金	自由申请项目	2016/6/1	2019/6/1	10
2016 年千人计划入选者省财政配套科研工作经费 10		杨克礼	广东省专项财政资金	千人计划	2016/9/6	2019/12/31	50
2016 年特支计划科研工作经费 18		周洁	广东省特支计划	百千万工程领军人才	2016/8/30	2018/12/31	50
2016 年特支计划科研工作经费 20		田国宝	广东省特支计划	百千万工程青年拔尖人才	2016/9/1	2018/8/31	10
基于岭南药用植物中寻找 RORγt 天然小分子抑制剂及其在狼疮性肾炎治疗中的应用	2016A050503023	黄朝峰	广东省科技计划	粤港联合创新领域	2016/1/1	2017/12/31	100
广东省-泰国重要螺传寄生虫病的防治研究	2016A050502008	吕志跃	广东省科技计划	科技计划项目	2016/5/1	2018/5/31	50
广东省寨卡病毒流行风险预警及应当研究（子项目）		郑小英	广东省科技计划	参与项目	2016/3/1	2018/2/28	30
登革病毒调控干扰素诱导的 MHC-I 表达和减弱 CD8+T 细胞功能的研究	2016A020219003	李义平	广东省科技计划	科技计划项目	2016/1/1	2017/12/31	30
Csseverin 在华支睾吸虫致肝胆管癌中的作用及机制研究	2016A020219004	黄艳	广东省科技计划	社会发展领域	2016/1/1	2018/12/31	30
动物和人源产碳青霉烯酶超级细菌的流行病学和传播机制比较研究	2016A020219002	田国宝	广东省科技计划	科技计划项目	2016/4/1	2018/12/31	30

“广东省特支计划”科技创新青年拔尖人才	2015TQ01R281	朱勋	广东省科技计划	广东特支计划-科技创新青年拔尖人才	2016/5/16	2019/7/31	30
“广东特支计划”科技青年拔尖人才	2015TQ01R473	吴敏昊	广东省科技计划	广东特支计划-科技创新青年拔尖人才	2016/6/1	2019/5/31	30
寨卡病毒病原学与免疫研究		黄曦	广东省科技计划	参与项目	2016/3/3	2017/3/3	9
抗耐药结核分枝杆菌 1.1 类新药 TB47 及新型结核病诊断产品的研发	201604020019	赖小敏	广州市科技计划	健康医疗协同创新重大专项参与	2016/5/23	2018/9/30	31.5
糖皮质激素诱导的肿瘤坏死因子受体 GITR 介导的抗结核免疫机制研究	201610010064	吴敏昊	广州市科技计划	创新人才培养计划-珠江科技新星专项	2016/5/1	2019/4/30	30
α-生育酚对日本血吸虫生长发育的抑制作用及其分子机制研究	2060404	吕志跃	广州市科技计划	科技专项	2016/1/1	2019/3/31	20
遗传性疾病综合检测技术的集成研发和规模应用	201604020091	蒋玮莹	广州市科技计划	参与项目	2016/4/20	2019/4/20	6
临床与转化医学国际合作联合实验室	50000-18823912	黎孟枫	高校基本业务费	校长经费	2016/9/1	2017/9/1	50
热带病防治研究教育部重点实验室	50000-18823910	黎孟枫	高校基本业务费	校长经费	2016/8/1	2017/8/1	50
热带病防治教育部重点实验室建设与发展		黎孟枫	高校基本业务费	其他	2016/7/1	2017/6/30	50
登革病毒调节宿主锌指蛋白家族介导天然免疫逃逸的分子机制研究	16ykzd16	朱勋	高校基本业务费	青年教师重点培育项目	2016/1/1	2017/12/31	30
耐碳青霉稀类药物“超级细菌”的耐药传播分子机制及临床治疗	16ykzd09	田国宝	高校基本业务费	青年教师重点培育项目	2016/3/22	2017/12/31	30
重要热带病传播相关的入侵媒介生物及其病原体的生物学特性研究-预研经费		吴忠道	医学科研管理基金	校内项目	2016/3/1	2017/2/28	10
热带重要新发突发病毒的感染与播散机制-预研经费		曾谷城	医学科研管理基金	校内项目	2016/3/1	2017/2/28	10
重要热带病传播相关的入侵媒介生物及其病原体的生物学特性研究-预研经费（第二批）		吴忠道	医学科研管理基金	校内项目	2016/8/1	2017/7/31	10

2016 年实验室发表论文

注：论文均标注重点实验室名称

序号	作者	论文题目	刊物，年，卷（期）：页码
1	Pan T, Zhong L, Wu S, Cao Y, Yang Q, Cai Z, Cai X, Zhao W, Ma N, Zhang W, Zhang H, Zhou J.	17β-Oestradiol enhances the expansion and activation of myeloid-derived suppressor cells via signal transducer and activator of transcription (STAT)-3 signalling in human pregnancy.	Clin Exp Immunol. 2016 Jul;185(1):86-97. doi: 10.1111/cei.12790.
2	Sun X, Wang LF, Feng Y, Xie H, Zheng XY, He A, Karim MR, Lv ZY, Wu ZD.	A case report: A rare case of infant gastrointestinal canthariasis caused by larvae of Lasioderma serricorne (Fabricius, 1792) (Coleoptera: Anobiidae).	Infect Dis Poverty. 2016 May 3;5:34. doi: 10.1186/s40249-016-0129-6.
3	Fang Z, Jiang C, Tang J, He M, Lin X, Chen X, Han L, Zhang Z, Feng Y, Guo Y, Li H, Jiang W.	A comprehensive analysis of membrane and morphology of erythrocytes from patients with glucose-6-phosphate dehydrogenase deficiency.	J Struct Biol. 2016 Jun;194(3):235-43. doi: 10.1016/j.jsb.2015.10.015.
4	Ye W, Lu B, Liu H.	A Rare Cause of Multiple Intestinal Mass Lesions.	Gastroenterology. 2016 Mar;150(3):570-1. doi: 10.1053/j.gastro.2015.11.052. No abstract available.
5	Pan T, Zhang Y, Zhou N, He X, Chen C, Liang L, Duan X, Lin Y, Wu K, Zhang H.	A recombinant chimeric protein specifically induces mutant KRAS degradation and potently inhibits pancreatic tumor growth.	Oncotarget. 2016 Jul 12;7(28):44299-44309. doi: 10.18632/oncotarget.9996.
6	Chen ZJ, Wei W, Jiang GM, Liu H, Wei WD, Yang X, Wu YM, Liu H, Wong CK, Du J, Wang HS.	Activation of GPER suppresses epithelial mesenchymal transition of triple negative breast cancer cells via NF-魏 B signals.	Mol Oncol. 2016 Jun;10(6):775-88. doi: 10.1016/j.molonc.2016.01.002.
7	Wu Y, Shi W, Lin J, Wang M, Chen X, Liu K, Xie Y, Luo L, Anderson BD, Lednicky JA, Gray GC, Lu J, Wang T.	Aerosolized avian influenza A (H5N6) virus isolated from a live poultry market, China.	J Infect. 2017 Jan;74(1):89-91. doi: 10.1016/j.jinf.2016.08.002. No abstract available.
8	Zhao BC, Jiang HY, Ma	Albendazole and Corticosteroids	PLoS Negl Trop Dis. 2016 Feb

	WY, Jin DD, Li HM, Lu H, Nakajima H, Huang TY, Sun KY, Chen SL, Chen KB.	for the Treatment of Solitary Cysticercus Granuloma: A Network Meta-analysis.	5;10(2):e0004418. doi: 10.1371/journal.pntd.0004418.
9	Lin RH, Lai DH, Zheng LL, Wu J, Lukeš J, Hide G, Lun ZR.	Analysis of the mitochondrial maxicircle of Trypanosoma lewisi, a neglected human pathogen.	Parasit Vectors. 2015 Dec 30;8:665. doi: 10.1186/s13071-015-1281-8.
10	Song L, Wang X, Yang Z, Lv Z, Wu Z.	Angiostrongylus cantonensis in the vector snails Pomacea canaliculata and Achatina fulica in China: a meta-analysis.	Parasitol Res. 2016 Mar;115(3):913-23. doi: 10.1007/s00436-015-4849-5.
11	Song T, Gan W, Chen J, Huang L, Yin H, He T, Huang H, Hu X.	Antibodies against Clonorchis sinensis LDH could cross-react with LDHB localizing on the plasma membrane of human hepatocarcinoma cell SMMC-7721 and induce apoptosis.	Parasitol Res. 2016 Apr;115(4):1595-603. doi: 10.1007/s00436-015-4895-z.
12	Pu J, He L, Xie H, Wu S, Li Y, Zhang P, Yang Z, Huang X.	Antiviral activity of Carbenoxolone disodium against dengue virus infection.	J Med Virol. 2016 May 7. doi: 10.1002/jmv.24571. [Epub ahead of print]
13	Ni Loo CS, Kei Lam NS, Yu D, Su XZ, Lu F.	Artemisinin and its derivatives in treating protozoan infections beyond malaria.	Pharmacol Res. 2016 Nov 17. pii: S1043-6618(16)30671-5. doi: 10.1016/j.phrs.2016.11.012. [Epub ahead of print] Review.
14	Song L, Wu X, Ren J, Gao Z, Xu Y, Xie H, Li D, Gong Z, Hu F, Liu H, Chen Y, Wu Z, Ning A.	Assessment of the effect of treatment and assistance program on advanced patients with schistosomiasis japonica in China from 2009 to 2014.	Parasitol Res. 2016 Nov;115(11):4267-4273.
15	Liu L, Guan H, Li Y, Ying Z, Wu J, Zhu X, Song L, Li J, Li M.	Astrocyte Elevated Gene-1 Interacts with Acetyltransferase p300 and c-Jun to Promote Tumor Aggressiveness.	Mol Cell Biol. 2016 Dec 12. pii: MCB.00456-16. [Epub ahead of print]
16	Lei A, Yang Q, Li X, Chen H, Shi M, Xiao Q, Cao Y, He Y, Zhou J.	Atorvastatin promotes the expansion of myeloid-derived suppressor cells and attenuates murine colitis.	Immunology. 2016 Dec;149(4):432-446. doi: 10.1111/imm.12662.
17	Anderson BD, Ma M, Xia Y, Wang T, Shu B, Lednicky JA, Ma MJ, Lu J, Gray GC.	Bioaerosol Sampling in Modern Agriculture: A Novel Approach for Emerging Pathogen Surveillance?	J Infect Dis. 2016 Aug 15;214(4):537-45. doi: 10.1093/infdis/jiw180.
18	Shen H, Wang F, Zeng G,	Bis-biguanide dihydrochloride	Sci Rep. 2016 Sep 7;6:32725.

	Shen L, Cheng H, Huang D, Wang R, Rong L, Chen ZW.	inhibits intracellular replication of M. tuberculosis and controls infection in mice.	doi: 10.1038/srep32725.
19	Liu J, Huang S, Su XZ, Song J, Lu F.	Blockage of Galectin-receptor Interactions by 伪-lactose Exacerbates Plasmodium berghei-induced Pulmonary Immunopathology.	Sci Rep. 2016 Aug 24;6:32024. doi: 10.1038/srep32024.
20	Long X, Li Y, Yang M, Huang L, Gong W, Kuang E.	BZLF1 Attenuates Transmission of Inflammatory Paracrine Senescence in Epstein-Barr Virus-Infected Cells by Downregulating Tumor Necrosis Factor Alpha.	J Virol. 2016 Aug 12;90(17):7880-93. doi: 10.1128/JVI.00999-16.
21	Song Q, Mao Y, Wilkins M, Segato F, Prade R	Cellulase immobilization on superparamagnetic nanoparticles for reuse in cellulosic biomass conversion	Aims Bioengineering. 2016. 3;(3): 264-276. doi: 10.3934/bioeng.2016.3.264
22	Liu J, Huang F, Zhang J, Tan L, Lu G, Zhang X, Zhang H.	Characteristic amino acid changes of influenza A(H1N1)pdm09 virus PA protein enhance A(H7N9) viral polymerase activity.	Virus Genes. 2016 Jun;52(3):346-53. doi: 10.1007/s11262-016-1311-4.
23	Chen D, Xie H, Cha H, Qu J, Wang M, Li L, Yu S, Wu C, Tang X, Huang J.	Characteristics of Schistosoma japonicum infection induced IFN-纬 and IL-4 co-expressing plasticity Th cells.	Immunology. 2016 Sep;149(1):25-34. doi: 10.1111/imm.12623.
24	Tian GB, Jiang YQ, Huang YM, Qin Y, Feng LQ, Zhang XF, Li HY, Zhong LL, Zeng KJ, Patil S, Xing Y, Huang X.	Characterization of CTX-M-140, a Variant of CTX-M-14 Extended-Spectrum beta-Lactamase with Decreased Cephalosporin Hydrolytic Activity, from Cephalosporin-Resistant Proteus mirabilis.	Antimicrob Agents Chemother. 2016 Sep 23;60(10):6121-6. doi: 10.1128/AAC.00822-16.
25	Liu B, Zou F, Lu L, Chen C, He D, Zhang X, Tang X, Liu C, Li L, Zhang H.	Chimeric Antigen Receptor T Cells Guided by the Single-Chain Fv of a Broadly Neutralizing Antibody Specifically and Effectively Eradicate Virus Reactivated from Latency in CD4+ T Lymphocytes Isolated from HIV-1-Infected Individuals Receiving Suppressive C	J Virol. 2016 Oct 14;90(21):9712-9724.

26	Yang M, Huang L, Li X, Kuang E.	Chloroquine inhibits lytic replication of Kaposi's sarcoma-associated herpesvirus by disrupting mTOR and p38-MAPK activation.	Antiviral Res. 2016 Sep;133:223-33. doi: 10.1016/j.antiviral.2016.08.010.
27	Lei AH, Yang Q, Cai WP, Liu YF, Lan Y, Qin AP, Hu FY, Zhou J.	Clinical Significance of Myeloid-Derived Suppressor Cells in Human Immunodeficiency Virus-1/ Hepatitis C Virus-coinfected Patients.	Scand J Immunol. 2016 Jun;83(6):438-44. doi: 10.1111/sji.12429.
28	Li W, Dong H, Huang Y, Chen T, Kong X, Sun H, Yu X, Xu J.	Clonorchis sinensis Co-infection Could Affect the Disease State and Treatment Response of HBV Patients.	PLoS Negl Trop Dis. 2016 Jun 27;10(6):e0004806. doi: 10.1371/journal.pntd.0004806.
29	Zhou L, Shang M, Shi M, Zhao L, Lin Z, Chen T, Wu J, Tang Z, Sun H, Yu J, Huang Y, Yu X.	Clonorchis sinensis lysophospholipase inhibits TGF-beta 1-induced expression of pro-fibrogenic genes through attenuating the activations of Smad3, JNK2, and ERK1/2 in hepatic stellate cell line LX-2	Parasitol Res. 2016 Feb;115(2):643-50.
30	Liao HY, Tao L, Zhao J, Qin J, Zeng GC, Cai SW, Li Y, Zhang J, Chen HG.	Clostridium butyricum in combination with specific immunotherapy converts antigen-specific B cells to regulatory B cells in asthmatic patients.	Sci Rep. 2016 Feb 9;6:20481. doi: 10.1038/srep20481.
31	Zhang GL, Zhang T, Ye YN, Liu J, Zhang XH, Xie C, Peng L, Gao ZL.	Complement Factor 3 Could Be an Independent Risk Factor for Mortality in Patients with HBV Related Acute-on-Chronic Liver Failure.	Biomed Res Int. 2016;2016:3524842. doi: 10.1155/2016/3524842.
32	He ZP, Dai XB, Zhang S, Zhi TT, Lun ZR, Wu ZD, Yang TB.	Complete mitochondrial genome of the giant African snail, Achatina fulica (Mollusca: Achatinidae): a novel location of putative control regions (CR) in the mitogenome within Pulmonate species.	Mitochondrial DNA A DNA Mapp Seq Anal. 2016;27(2):1084-5. doi: 10.3109/19401736.2014.930833.
33	Wu Y, Jie Y, Li X, Huang M, Li X, Shi H, Chen S, Zhang M, Ao Y, Yang F, Gao Z, Lin G, Chong Y.	Comprehensive analysis of hospital-based prospective cohort reveals the unique effectiveness and safety for nucleos(t)ide	Clin Res Hepatol Gastroenterol. 2016 Apr;40(2):213-20. doi: 10.1016/j.clinre.2015.06.018.

		analogues in HBV patients.	
34	Liang W, Xie Z, Cui W, Guo Y, Xu L, Wu J, Guan H.	Comprehensive gene and microRNA expression profiling reveals a role for miRNAs in the oncogenic roles of SphK1 in papillary thyroid cancer.	J Cancer Res Clin Oncol. 2016 Dec 10. [Epub ahead of print]
35	Zhong LL, Zhang YF, Doi Y, Huang X, Zhang XF, Zeng KJ, Shen C, Patil S, Xing Y, Zou Y, Tian GB.	Co-production of MCR-1 and NDM-1 by colistin-resistant Escherichia coli isolated from a healthy individual.	Antimicrob Agents Chemother. 2016 Nov 7. pii: AAC.01962-16. [Epub ahead of print]
36	Tang ZL, Huang Y, Yu XB.	Current status and perspectives of Clonorchis sinensis and clonorchiasis: epidemiology, pathogenesis, omics, prevention and control.	Infect Dis Poverty. 2016 Jul 6;5(1):71. doi: 10.1186/s40249-016-0166-1. Review.
37	Lai DH, Hong XK, Su BX, Liang C, Hide G, Zhang X, Yu X, Lun ZR.	Current status of Clonorchis sinensis and clonorchiasis in China.	Trans R Soc Trop Med Hyg. 2016 Jan;110(1):21-7. doi: 10.1093/trstmh/trv100. Review.
38	He Z, Zhu X, Wen W, Yuan J, Hu Y, Chen J, An S, Dong X, Lin C, Yu J, Wu J, Yang Y, Cai J, Li J, Li M.	Dengue Virus Subverts Host Innate Immunity by Targeting Adaptor Protein MAVS.	J Virol. 2016 Jul 27;90(16):7219-30. doi: 10.1128/JVI.00221-16.
39	Zhang Y, Wang T, Liu K, Xia Y, Lu Y, Jing Q, Yang Z, Hu W, Lu J.	Developing a Time Series Predictive Model for Dengue in Zhongshan, China Based on Weather and Guangzhou Dengue Surveillance Data.	PLoS Negl Trop Dis. 2016 Feb 19;10(2):e0004473. doi: 10.1371/journal.pntd.0004473.
40	Geng G, Liu B, Chen C, Wu K, Liu J, Zhang Y, Pan T, Li J, Yin Y, Zhang J, Huang F, Yu F, Chen J, Ma X, Zhou J, Kuang E, Liu C, Cai W, Zhang H.	Development of an Attenuated Tat Protein as a Highly-effective Agent to Specifically Activate HIV-1 Latency.	Mol Ther. 2016 Sep;24(9):1528-37. doi: 10.1038/mt.2016.117.
41	Fang Z, Jiang C, Feng Y, Chen R, Lin X, Zhang Z, Han L, Chen X, Li H, Guo Y, Jiang W.	Effects of G6PD activity inhibition on the viability, ROS generation and mechanical properties of cervical cancer cells.	Biochim Biophys Acta. 2016 Sep;1863(9):2245-54. doi: 10.1016/j.bbamcr.2016.05.016.
42	Yiwen Z, Shilin G, Yingshi C, Lishi S, Baohong L, Chao L, Linghua L, Ting P, Hui Z.	Efficient generation of antigen-specific CTLs by the BAFF-activated human B Lymphocytes as APCs: a novel approach for immunotherapy.	Oncotarget. 2016 Nov 22;7(47):77732-77748. doi: 10.18632/oncotarget.12792.
43	Zeng KJ, Doi Y, Patil S,	Emergence of the	Antimicrob Agents Chemother.

	Huang X, Tian GB.	Plasmid-Mediated mcr-1 Gene in Colistin-Resistant Enterobacter aerogenes and Enterobacter cloacae.	2016 May 23;60(6):3862-3. doi: 10.1128/AAC.00345-16. No abstract available.
44	Yin Y, Xu Y, Su L, Zhu X, Chen M, Zhu W, Xia H, Huang X, Gong S.	Epidemiologic investigation of a family cluster of imported ZIKV cases in Guangdong, China: probable human-to-human transmission.	Emerg Microbes Infect. 2016 Sep 7;5(9):e100. doi: 10.1038/emi.2016.100.
45	Cai W, Lu C, Li X, Zhang J, Zhan P, Xi L, Sun J, Yu X.	Epidemiology of Superficial Fungal Infections in Guangdong, Southern China: A Retrospective Study from 2004 to 2014.	Mycopathologia. 2016 Jun;181(5-6):387-95. doi: 10.1007/s11046-016-9986-6.
46	Li Y, Long X, Huang L, Yang M, Yuan Y, Wang Y, Delecluse HJ, Kuang E.	Epstein-Barr Virus BZLF1-Mediated Downregulation of Proinflammatory Factors Is Essential for Optimal Lytic Viral Replication	J Virol. 2015 Nov 4;90(2):887-903. doi: 10.1128/JVI.01921-15.
47	Lin RH, Lai DH, Zheng LL, Wu J, Luke 拧 J, Hide G, Lun ZR.	Erratum to: Analysis of the mitochondrial maxicircle of Trypanosoma lewisi, a neglected human pathogen.	Parasit Vectors. 2016 Jan 13;9:15. doi: 10.1186/s13071-016-1297-8. No abstract available.
48	Zhang S, Wu K, Liu Y, Lin Y, Zhang X, Zhou J, Zhang H, Pan T, Fu Y.	Finasteride Enhances the Generation of Human Myeloid-Derived Suppressor Cells by Up-Regulating the COX2/PGE2 Pathway.	PLoS One. 2016 Jun 2;11(6):e0156549. doi: 10.1371/journal.pone.0156549.
49	Li YP, Van Pham L, Uzcategui N, Bukh J.	Functional analysis of microRNA-122 binding sequences of hepatitis C virus and identification of variants with high resistance against a specific antagomir.	J Gen Virol. 2016 Jun;97(6):1381-94. doi: 10.1099/jgv.0.000445.
50	Liu YF, Wei JY, Shi MH, Jiang H, Zhou J.	Glucocorticoid Induces Hepatic Steatosis by Inhibiting Activating Transcription Factor 3 (ATF3)/S100A9 Protein Signaling in Granulocytic Myeloid-derived Suppressor Cells.	J Biol Chem. 2016 Oct 7;291(41):21771-21785.
51	Zhou N, Pan T, Zhang J, Li Q, Zhang X, Bai C, Huang F, Peng T, Zhang J, Liu C,	Glycopeptide Antibiotics Potently Inhibit Cathepsin L in the Late Endosome/Lysosome	J Biol Chem. 2016 Apr 22;291(17):9218-32. doi: 10.1074/jbc.M116.716100.

	Tao L, Zhang H.	and Block the Entry of Ebola Virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV).	
52	Serre SB, Jensen SB, Ghanem L, Humes DG, Ramirez S, Li YP, Krarup H, Bukh J, Gottwein JM.	Hepatitis C Virus Genotype 1 to 6 Protease Inhibitor Escape Variants In Vitro Selection, Fitness, and Resistance Patterns in the Context of the Infectious Viral Life	Antimicrob Agents Chemother. 2016 May 23;60(6):3563-78. doi: 10.1128/AAC.02929-15. Print 2016 Jun.
53	Haq SZ, Abushahama MS, Gerwash O, Hughes JM, Wright EA, Elmahaishi MS, Lun ZR, Thomasson D, Hide G.	High frequency detection of Toxoplasma gondii DNA in human neonatal tissue from Libya.	Trans R Soc Trop Med Hyg. 2016 Sep;110(9):551-557.
54	Song LG, Wu XY, Sacko M, Wu ZD.	History of schistosomiasis epidemiology, current status, and challenges in China: on the road to schistosomiasis elimination.	Parasitol Res. 2016 Nov;115(11):4071-4081. Review.
55	Zhang Y, Yin Y, Zhang S, Luo H, Zhang H.	HIV-1 Infection-Induced Suppression of the Let-7i/IL-2 Axis Contributes to CD4(+) T Cell Death.	Sci Rep. 2016 May 5;6:25341. doi: 10.1038/srep25341.
56	Zhang J, Huang F, Tan L, Bai C, Chen B, Liu J, Liang J, Liu C, Zhang S, Lu G, Chen Y, Zhang H.	Host Protein Moloney Leukemia Virus 10 (MOV10) Acts as a Restriction Factor of Influenza A Virus by Inhibiting the Nuclear Import of the Viral Nucleoprotein.	J Virol. 2016 Mar 28;90(8):3966-80. doi: 10.1128/JVI.03137-15.
57	Chi X, Niu Y, Cheng M, Liu X, Feng Y, Zheng F, Fan J, Li X, Jin Q, Zhong J, Li YP, Yang W.	Identification of a Potent and Broad-Spectrum Hepatitis C Virus Fusion Inhibitory Peptide from the E2 Stem Domain.	Sci Rep. 2016 Apr 28;6:25224. doi: 10.1038/srep25224.
58	Peng H, Zhang Q, Li X, Liu Z, Shen J, Sun R, Wei J, Zhao J, Wu X, Feng F, Zhong S, Sun X, Wu Z.	IL-33 Contributes to Schistosoma japonicum-induced Hepatic Pathology through Induction of M2 Macrophages.	Sci Rep. 2016 Jul 21;6:29844. doi: 10.1038/srep29844.
59	Zhang X, He X, Liu C, Liu J, Hu Q, Pan T, Duan X, Liu B, Zhang Y, Chen J, Ma X, Zhang X, Luo H, Zhang H.	IL-4 Inhibits the Biogenesis of an Epigenetically Suppressive PIWI-Interacting RNA To Upregulate CD1a Molecules on Monocytes/Dendritic Cells.	J Immunol. 2016 Feb 15;196(4):1591-603. doi: 10.4049/jimmunol.1500805.

60	Jiang H, Chen T, Sun H, Tang Z, Yu J, Lin Z, Ren P, Zhou X, Huang Y, Li X, Yu X.	Immune response induced by oral delivery of Bacillus subtilis spores expressing enolase of Clonorchis sinensis in grass carps (Ctenopharyngodon idellus).	Fish Shellfish Immunol. 2016 Oct 8;60:318-325. doi: 10.1016/j.fsi.2016.10.011. [Epub ahead of print]
61	Xiao S, Liu J, Huang S, Lu F.	Increased Gal-9 and Tim-3 expressions during liver damage in a murine malarial model	Parasitol Res. 2016 Feb;115(2):663-72.
62	Zhong J, Huang W, Deng Q, Wu M, Jiang H, Lin X, Sun Y, Huang X, Yuan J.	Inhibition of TREM-1 and Dectin-1 Alleviates the Severity of Fungal Keratitis by Modulating Innate Immune Responses.	PLoS One. 2016 Mar 10;11(3):e0150114. doi: 10.1371/journal.pone.0150114.
63	Bian M, Xu Q, Xu Y, Li S, Wang X, Sheng J, Wu Z, Huang Y, Yu X.	Investigation on oxidative stress of nitric oxide synthase interacting protein from Clonorchis sinensis.	Parasitol Res. 2016 Jan;115(1):77-83. doi: 10.1007/s00436-015-4723-5.
64	Zheng S, Zhu Y, Zhao Z, Wu Z, Okanurak K, Lv Z.	Liver fluke infection and cholangiocarcinoma: a review.	Parasitol Res. 2017 Jan;116(1):11-19. Review.
65	Li J, Chen C, Ma X, Geng G, Liu B, Zhang Y, Zhang S, Zhong F, Liu C, Yin Y, Cai W, Zhang H.	Long noncoding RNA NRON contributes to HIV-1 latency by specifically inducing tat protein degradation.	Nat Commun. 2016 Jun 13;7:11730. doi: 10.1038/ncomms11730.
66	Yang K, Wu Y, Xie H, Li M, Ming S, Li L, Li M, Wu M, Gong S, Huang X.	Macrophage-mediated inflammatory response decreases mycobacterial survival in mouse MSCs by augmenting NO production.	Sci Rep. 2016 Jun 2;6:27326. doi: 10.1038/srep27326.
67	Li S, Mao Y, Zhou T, Luo C, Xie J, Qi W, Yang Z, Ma J, Gao G, Yang X.	Manganese superoxide dismutase mediates anoikis resistance and tumor metastasis in nasopharyngeal carcinoma.	Oncotarget. 2016 May 31;7(22):32408-20. doi: 10.18632/oncotarget.8717.
68	Diao D, Wang L, Wan J, Chen Z, Peng J, Liu H, Chen X, Wang W, Zou L.	MEK5 overexpression is associated with the occurrence and development of colorectal cancer.	BMC Cancer. 2016 May 9;16:302. doi: 10.1186/s12885-016-2327-9.
69	Xu F, Li X, Zhang P, Xia J, Wang Y, Yang C, Gao H, Zhou S, Zhang P.	Melanoma differentiation-associated gene 5 is involved in the induction of stress granules and autophagy by protonophore CCCP	Biol Chem. 2016 Jan;397(1):67-74. doi: 10.1515/hsz-2015-0195.
70	Fu X, Yu S, Yang B, Lao S, Li B, Wu C.	Memory-Like Antigen-Specific Human NK Cells from TB Pleural Fluids Produced IL-22 in	PLoS One. 2016 Mar 31;11(3):e0151721. doi: 10.1371/journal.pone.0151721.

		Response to IL-15 or Mycobacterium tuberculosis Antigens.	
71	Zhou T, Che D, Lan Y, Fang Z, Xie J, Gong H, Li C, Feng J, Hong H, Qi W, Ma C, Yang Z, Cai W, Zhong J, Ma J, Yang X, Gao G.	Mesenchymal marker expression is elevated in Müller cells exposed to high glucose and in animal models of diabetic retinopathy.	Oncotarget. 2016 Dec 15. doi: 10.18632/oncotarget.13945. [Epub ahead of print]
72	Li M, Wang J, Fang Y, Gong S, Li M, Wu M, Lai X, Zeng G, Wang Y, Yang K, Huang X.	microRNA-146a promotes mycobacterial survival in macrophages through suppressing nitric oxide production.	Sci Rep. 2016 Mar 30;6:23351. doi: 10.1038/srep23351.
73	Wang L, Wu J, Yuan J, Zhu X, Wu H, Li M.	Midline2 is overexpressed and a prognostic indicator in human breast cancer and promotes breast cancer cell proliferation in vitro and in vivo.	Front Med. 2016 Mar;10(1):41-51. doi: 10.1007/s11684-016-0429-z.
74	Wang P, Chen S, Fang H, Wu X, Chen D, Peng L, Gao Z, Xie C.	miR-214/199a/199a* cluster levels predict poor survival in hepatocellular carcinoma through interference with cell-cycle regulators.	Oncotarget. 2016 Jan 5;7(1):929-45. doi: 10.18632/oncotarget.6137.
75	Li L, Jiang Y, Lao S, Yang B, Yu S, Zhang Y, Wu C.	Mycobacterium tuberculosis-Specific IL-21+IFN-纬+CD4+ T Cells Are Regulated by IL-12.	PLoS One. 2016 Jan 19;11(1):e0147356. doi: 10.1371/journal.pone.0147356.
76	Pan T, Liu Y, Zhong LM, Shi MH, Duan XB, Wu K, Yang Q, Liu C, Wei JY, Ma XR, Shi K, Zhang H, Zhou J.	Myeloid-derived suppressor cells are essential for maintaining feto-maternal immunotolerance via STAT3 signaling in mice.	J Leukoc Biol. 2016 Sep;100(3):499-511. doi: 10.1189/jlb.1A1015-481RR.
77	Huang L, Yang M, Yuan Y, Li X, Kuang E.	Niclosamide inhibits lytic replication of Epstein-Barr virus by disrupting mTOR activation.	Antiviral Res. 2016 Dec 8;138:68-78. doi: 10.1016/j.antiviral.2016.12.002. [Epub ahead of print]
78	Zhao M, Chen H, Ding Q, Xu X, Yu B, Huang Z.	Nuclear Factor Erythroid 2-related Factor 2 Deficiency Exacerbates Lupus Nephritis in B6/lpr mice by Regulating Th17 Cell Function.	Sci Rep. 2016 Dec 12;6:38619. doi: 10.1038/srep38619.
79	He P, Song LG, Xie H, Liang JY, Yuan DY, Wu	Nucleic acid detection in the diagnosis and prevention of	Infect Dis Poverty. 2016 Mar 30;5:25. doi:

	ZD, Lv ZY.	schistosomiasis.	10.1186/s40249-016-0116-y. Review.
80	Wu J, Liu L, Wang G, Lu J.	One Health in China.	Infect Ecol Epidemiol. 2016 Nov 29;6:33843. doi: 10.3402/iee.v6.33843. Review.
81	Zhang G, Zhang T, Zhao Q, Xie C, Liu J, Peng L, Gao Z.	Overproduction of IL-27 may play a pro-inflammatory role in HBV infected patients with severe liver inflammation	International Journal of Clinical and Experimental Medicine. 2016. 9(3): 7000-7007.
82	Xiao L, Jia L, Bai L, He L, Yang B, Wu C, Li H.	Phenotypic and functional characteristics of IL-21-expressing CD8(+) T cells in human nasal polyps.	Sci Rep. 2016 Jul 29;6:30362. doi: 10.1038/srep30362.
83	Zhang XF, Doi Y, Huang X, Li HY, Zhong LL, Zeng KJ, Zhang YF, Patil S, Tian GB.	Possible Transmission of mcr-1-Harboring Escherichia coli between Companion Animals and Human	Emerg Infect Dis. 2016 Sep;22(9):1679-81. doi: 10.3201/eid2209.160464. Epub 2016 Sep 15.
84	Wen C, Chen J, Zhang D, Wang H, Che J, Qin Q, He L, Cai Z, Lin M, Lou Q, Huang L, Chen D, Iwamoto A, Ren D, Wang L, Lan P, Wang J, Liu H, Yang X.	Pseudolaric acid B induces mitotic arrest and apoptosis in both 5-fluorouracil-sensitive and -resistant colorectal cancer cells.	Cancer Lett. 2016 Dec 28;383(2):295-308. doi: 10.1016/j.canlet.2016.09.007.
85	Wu Y, Li D, Wang Y, Chen K, Yang K, Huang X, Zhang Y, Wu M.	Pseudomonas aeruginosa promotes autophagy to suppress macrophage-mediated bacterial eradication.	Int Immunopharmacol. 2016 Sep;38:214-22. doi: 10.1016/j.intimp.2016.04.044.
86	Deng Q, Wang Y, Zhang Y, Li M, Li D, Huang X, Wu Y, Pu J, Wu M.	Pseudomonas aeruginosa Triggers Macrophage Autophagy To Escape Intracellular Killing by Activation of the NLRP3 Inflammasome	Infect Immun. 2015 Oct 14;84(1):56-66. doi: 10.1128/IAI.00945-15.
87	Sun X, Yang F, Shen J, Liu Z, Liang J, Zheng H, Fung M, Wu Z.	Recombinant Sj16 from Schistosoma japonicum contains a functional N-terminal nuclear localization signal necessary for nuclear translocation in dendritic cells and interleukin-10 production.	Parasitol Res. 2016 Dec;115(12):4559-4571.
88	Liu KK, Wang T, Huang XD, Wang GL, Xia Y, Zhang YT, Jing QL, Huang JW, Liu XX, Lu JH, Hu WB.	Risk assessment of dengue fever in Zhongshan, China: a time-series regression tree analysis.	Epidemiol Infect. 2016 Nov 22:1-11. [Epub ahead of print]

89	Song T, Chen J, Huang L, Gan W, Yin H, Jiang J, He T, Huang H, Hu X.	Should we abandon quinine plus antibiotic for treating uncomplicated falciparum malaria? A systematic review and meta-analysis of randomized controlled trials.	Parasitol Res. 2016 Mar;115(3):903-12. doi: 10.1007/s00436-015-4842-z. Review.
90	Wu F, Wei J, Liu Z, Zeng X, Yu Z, Lv Z, Sun X, Wu Z.	Soluble antigen derived from IV larva of Angiostrongylus cantonensis promotes chitinase-like protein 3 (Chil3) expression induced by interleukin-13.	Parasitol Res. 2016 Oct;115(10):3737-46. doi: 10.1007/s00436-016-5135-x.
91	Liu L, Wu S, Yang Y, Cai J, Zhu X, Wu J, Li M, Guan H.	SOSTDC1 is down-regulated in non-small cell lung cancer and contributes to cancer cell proliferation.	Cell Biosci. 2016 Apr 14;6:24. doi: 10.1186/s13578-016-0091-9.
92	Liu Z, Wu Y, Feng Y, Wu F, Liu RF, Wang LF, Liang JY, Liu JH, Sun X, Wu ZD.	Spleen atrophy related immune system changes attributed to infection of Angiostrongylus cantonensis in mouse model.	Parasitol Res. 2016 Nov 22. [Epub ahead of print]
93	Liu S, Chen L, Zeng Y, Si L, Guo X, Zhou J, Fang D, Zeng G, Jiang L.	Suppressed expression of miR-378 targeting gzmb in NK cells is required to control dengue virus infection.	Cell Mol Immunol. 2016 Sep;13(5):700-8. doi: 10.1038/cmi.2015.52.
94	Song T, Yin H, Chen J, Huang L, Jiang J, He T, Huang H, Hu X.	Survival advantage depends on cecal volume rather than cecal length in a mouse model of cecal ligation and puncture.	J Surg Res. 2016 Jun 15;203(2):476-82. doi: 10.1016/j.jss.2016.03.019.
95	Du Y, Liu L, Wang C, Kuang B, Yan S, Zhou A, Wen C, Chen J, Wu Y, Yang X, Feng G, Liu B, Iwamoto A, Zeng M, Wang J, Zhang X, Liu H.	TACC3 promotes colorectal cancer tumourigenesis and correlates with poor prognosis.	Oncotarget. 2016 Jul 5;7(27):41885-41897. doi: 10.18632/oncotarget.9628.
96	Sheng SY, Gu Y, Lu CG, Tang YY, Zou JY, Zhang YQ, Wang RF, Hong H.	The Characteristics of Naive-like T Cells in Tumor-infiltrating Lymphocytes From Human Lung Cancer.	J Immunother. 2017 Jan;40(1):1-10.
97	Hong H, Gu Y, Sheng SY, Lu CG, Zou JY, Wu CY.	The Distribution of Human Stem Cell-like Memory T Cell in Lung Cancer.	J Immunother. 2016 Jul-Aug;39(6):233-40. doi: 10.1097/CJI.0000000000000128.
98	Tang Z, Shang M, Chen T, Ren P, Sun H, Qu H, Lin Z, Zhou L, Yu J, Jiang H,	The immunological characteristics and probiotic function of recombinant Bacillus	Parasit Vectors. 2016 Dec 19;9(1):648.

	Zhou X, Li X, Huang Y, Xu J, Yu X.	subtilis spore expressing Clonorchis sinensis cysteine protease.	
99	Liu L, Wang T, Lu J.	The prevalence, origin, and prevention of six human coronaviruses.	Virol Sin. 2016 Feb;31(1):94-9. doi: 10.1007/s12250-015-3687-z. No abstract available.
100	Zhu X, Xie C, Li YM, Huang ZL, Zhao QY, Hu ZX, Wang PP, Gu YR, Gao ZL, Peng L.	TMEM2 inhibits hepatitis B virus infection in HepG2 and HepG2.2.15 cells by activating the JAK-STAT signaling pathway.	Cell Death Dis. 2016 Jun 2;7(6):e2239. doi: 10.1038/cddis.2016.146.
101	Jiang H, Si Y, Li Z, Huang X, Chen S, Zheng Y, Xu G, Chen X, Chen Y, Liu Y, Xiong H, Huang Q, Liang M, Zhang Z.	TREM-2 promotes acquired cholesteatoma-induced bone destruction by modulating TLR4 signaling pathway and osteoclasts activation.	Sci Rep. 2016 Dec 9;6:38761. doi: 10.1038/srep38761.
102	Zhou Q, Gu J, Lun ZR, Ayala FJ, Li Z.	Two distinct cytokinesis pathways drive trypanosome cell division initiation from opposite cell ends.	Proc Natl Acad Sci U S A. 2016 Mar 22;113(12):3287-92. doi: 10.1073/pnas.1601596113.
103	Liu J, Xiao S, Huang S, Pei F, Lu F.	Upregulated Tim-3/galectin-9 expressions in acute lung injury in a murine malarial model	Parasitol Res. 2016 Feb;115(2):587-95.
104	Peng J, Lin X, Lin H, Chen S, Liu J, Guo Z, Liang Y, Huang S, Lu F.	Up-regulated TLR2 and TLR4 expressions in liver and spleen during acute murine T. gondii infection.	Parasitol Res. 2016 Dec;115(12):4681-4686.
105	Liu K, Wang T, Yang Z, Huang X, Milinovich GJ, Lu Y, Jing Q, Xia Y, Zhao Z, Yang Y, Tong S, Hu W, Lu J.	Using Baidu Search Index to Predict Dengue Outbreak in China.	Sci Rep. 2016 Dec 1;6:38040. doi: 10.1038/srep38040.
106	Ma Y, Li X, Kuang E.	Viral Evasion of Natural Killer Cell Activation.	Viruses. 2016 Apr 12;8(4):95. doi: 10.3390/v8040095. Review.
107	Feng Q, He Y, Lu J.	Virus-Like Particles Produced in Pichia Pastoris Induce Protective Immune Responses Against Coxsackievirus A16 in Mice	Med Sci Monit. 2016 Sep 23;22:3370-3382.
108	李彤、刘彤、刘怡、赵龙、吕志跃	弓形虫感染对宿主神经系统影响的研究进展	热带医学杂志, 2016, 16(12): 1585-1587.
109	王梦、王娜娜、吕志跃	脑型疟疾的病因、症状和诊治进展	热带医学杂志, 2016 (2016 年 11): 1465-1467, 1479.
110	方丹云、郭晓兰、于玉凤、晏辉钧、司露露、王颖、	轻症和重症人感染 H7N9 禽流感病毒的致病性	中山大学学报: 医学科学版, 2016, 37(6): 828-833

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111	张梦颖、吕志跃、吴忠道	细胞凋亡发生机制研究进展	热带医学杂志, 2016 (2016 年 10): 1346-1349, 1352.
112	杨旭然、陈庭金、余新炳	诱导型一氧化氮合酶与寄生虫感染的研究进展	中国病原生物学杂志, 2016 (9): 855-857.
113	朱燕珊、陆家海、郭中敏	寨卡病毒的流行状况及其潜在问题	热带医学杂志, 2016, 16(9): 1214-1218.
114	陶海燕、潘中哲、潘茂林、卓莉、徐勇、鹿苗	广州大都市登革热时空传播混合模式	地理学报, 2016, 71(9): 1653-1662.
115	侯珠秀、陈庭金、黄艳、余新炳	IL-25 在寄生虫感染性疾病中的研究进展	热带医学杂志, 2016, 16(8): 1086-1088.
116	侯永恒、郝桃方、廖婉琴、杨兆收、潘爱华、吴忠道、周兴旺	弓形虫 ROP18 蛋白激酶在酵母中的表达纯化及激酶活性鉴定	热带医学杂志, 2016 (2016 年 06): 687-690
117	陈英安、马蒙蒙、张应涛、陆家海	广东养猪从业人员猪流感 H3、H1 亚型既往感染及其与猪场规模的关系	热带医学杂志, 2016 (2016 年 06): 799-802,817.
118	申东梅、方毅敏、申雁鸣、刘国标、姚亚男、赖小敏	结核特异性多肽 E6、E7 和 C14 对单核-巨噬细胞亚型极化的影响	中国医药生物技术, 2016 (3): 216-223.
119	陆诗淼、吴永坚、黄曦	TREM-2 在 DSS 诱导小鼠肠炎中的作用	热带医学杂志, 2016, 16(5): 547-550.
120	司露露、郭晓兰、郭鹏娟、王颖、罗雅艳、崔国辉、方丹云、周俊梅、晏辉钧、江丽芳	登革病毒 prM 抗体的中和作用及 ADE 作用研究	中山大学学报: 医学科学版, 2016, 37(3): 321-325
121	杨方集、吴元凯、陈淑如、谢茜、李新华、朱昌浩、谢冬英、崇雨田	登革热患者血小板减少与血小板膜糖蛋白特异性抗体相关	中山大学学报: 医学科学版, 2016, 37(3): 326-332
122	张素粉、徐霖、罗虹娇、庹玖玲、张甜、曹开源	2012-2015 年广州地区人冠状病毒及其亚型流行病学特征	热带医学杂志, 2016, 16(4): 430-433
123	陈英安、景钦隆、杨勤华、张应涛、杨智聪、陆家海	血小板计数、白细胞总数与登革热临床类型的相关性	热带医学杂志, 2016, 16(4): 523-526
124	杨宜英、黄艳君、谭耀驹、杨锟、田国宝、黄曦	T-SPOT.TB 在菌阳和菌阴结核病诊断中的应用价值	热带医学杂志, 2016, 16(4): 411-414.
125	李茜、吕芳丽	眼弓形虫病的研究进展	热带医学杂志, 2016, 16(3): 404-408.
126	Kalawati Shrestha、张应涛、Prem Raj Shrestha、张文东、刘丽珊、Pramod Koirala、Mohan Krishna Maharjan、陆家海	广州市黄埔区与加德满都谷地食品安全监管模式及现状的对比研究（英文）	热带医学杂志, 2016, 16(3): 193-330
127	郭鹏娟、詹希美、吴德、许少洪、黄芳、谭锦花	登革热病毒感染后 NS1 抗原检测的应用评价	中国卫生检验杂志, 2016 (5): 697-699.

128	任鹏丽、陈庭金、黄艳、曾伟伟、余新炳	草鱼呼肠孤病毒 HZ08 株外衣壳蛋白 VP4 的节段克隆表达和免疫学分析	热带医学杂志, 2016, 16(2): 140-144.
129	陈胜杰、吕芳丽	肥大细胞在原虫感染免疫中的作用研究进展	热带医学杂志, 2016, 16(2): 265-269.
130	刘均如、徐霖、钟慧玲、张素粉、罗洪娇、张定梅、张甜、曹开源	2010-2012 年广州地区腹泻患者中博卡病毒分子进化分析	热带医学杂志, 2016, 16(2): 149-152
131	聂恩琼、夏尧、汪涛、陆家海	One Health——应对新发传染病的新理念	微生物与感染, 2016, 11(1): 3-7.

2016 年实验室申请与授权的发明专利

申请专利

序号	专利名称	发明人	专利号
1	一种 VC-CAR 分子及在清除 HIV-1 感染细胞中的应用	张辉;刘炳峰;邹帆	201610652995.9
2	一种干细胞样记忆性 T 细胞体外诱导剂及方法	张辉;张译文	201610870886.4
3	夫西地酸或其药用盐在制备抗手足口病药物中的应用	曾施暖;郭学敏	201610008433
4	2-呋喃丙烯醛类化合物在制备抗人类γ疱疹病毒药物中的应用	徐峻;袁岩;徐梦阳;钟灿榕	201611209962.3
5	基于激光和图像处理的车辆侧倾角动态测量方法及装置	张辉;杨永强;庄文盛;龚文森	201610100230.4
6	一种车辆速度和位置的检测方法和装置	张辉;庄文盛;杨永强;龚文森	201610100342.X
7	一种芸香苦素类化合物及其在制备抗 EBV 药物中的应用	徐峻;林永胜;王倩;顾琼;袁岩;江程	201610262207.5
8	氯硝柳胺在制备抗致瘤疱疹病毒药物中的应用	况二胜;黄璐;杨梦甜	201610508580.4
9	可溶性蛋白 BAFF 在 B 细胞体外培养及扩增的应用	张辉;张译文;高士麟	201610692755.1
10	白介素-21 在制备干细胞样记忆 T 细胞体外扩增诱导剂中的应用	张辉;张译文;陈颖诗	201610826488.2

授权专利

序号	专利名称	发明人	专利号
1	二氧代咪唑烷-酰胺类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410231970.2
2	氰基-吡啶类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410232592.X
3	1-（2-氯苯基）-4-{噻吩并[3,2-d]嘧啶-4-基}哌嗪化合物在制备抗 HIV-1 病毒药物中应用	张辉;潘婷;罗海华;张旭;柏川	CN201410090801.1
4	噻吩-羧酸酯类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;柏川	CN201410104029.4
5	吡啶-哌嗪类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410231992.9
6	卤代呋喃类化合物在制备抗	张辉;潘婷;罗海华;张旭;何欣	CN201410231968.5

	HIV-1 病毒药物中的应用		
7	萘-吡啶类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410236332.X
8	一种增强基因表达的激活型 siRNA	张辉; 张意军; 樊苗苗	201410102029.0
9	苯乙烯基-磺酰胺类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410232555.9
10	一种 Tat 蛋白及其制备方法和应用	张辉; 耿冠男	201410259996.8
11	呋喃-羧酸酯类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;柏川	CN201410091181.3
12	苯并六元杂环类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410232733.8
13	酰氨基-噻吩类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410232708.X
14	苯基-酰胺类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410231868.2
15	一种抗病毒化合物在制备抗 HIV-1 病毒药物中的应用	张辉;柏川;潘婷	CN201410120209.1
16	苯-磺酰胺类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;柏川	CN201410103000.4
17	一种抗病毒化合物在制备抗 HIV-1 病毒药物中的应用	张辉;柏川;潘婷	CN201410120259.X
18	苯并五元杂环类化合物在制备抗 HIV-1 病毒药物中的应用	张辉;潘婷;罗海华;张旭;何欣	CN201410232554.4
19	一种抗病毒化合物在制备抗 HIV-1 病毒药物中的应用	张辉;柏川;潘婷	CN201410120208.7

2016 年实验室获得国家医疗器械证书

序号	产品名称	注册号	生产单位
1	柯萨奇病毒 A6 型核酸检测试剂盒（PCR-荧光探针法）	国械注准 20163401688	中山大学达安基因股份有限公司
2	结核分枝杆菌异烟肼耐药基因突变检测试剂盒（PCR-测序法）	国械注准 20163401687	中山大学达安基因股份有限公司
3	β-地中海贫血基因分型检测试剂盒（PCR-反向点杂交法）	国械注准 20163402013	中山大学达安基因股份有限公司
4	人类免疫缺陷病毒 1 型核酸定量检测试剂盒（巢式 PCR-荧光法）	国械注准 20163401041	中山大学达安基因股份有限公司
5	乙型肝炎病毒核酸检测试剂盒(PCR-荧光探针法)	国械注准 20163401028	中山大学达安基因股份有限公司
6	沙眼衣原体核酸检测试剂盒(PCR-荧光探针法)	国械注准 20163401027	中山大学达安基因股份有限公司
7	解脲脲原体基因分型检测试剂盒(PCR-反向点杂交法)	国械注准 20163400916	中山大学达安基因股份有限公司
8	淋球菌核酸测定试剂盒(PCR-荧光探针法)	国械注准 20163400962	中山大学达安基因股份有限公司
9	糖类抗原 CA15-3 测定试剂盒(免疫荧光法)	国械注准 20163400530	中山大学达安基因股份有限公司
10	单纯疱疹病毒Ⅱ型核酸测定试剂盒(PCR-荧光探针法)	国械注准 20163400532	中山大学达安基因股份有限公司
11	神经元特异性烯醇化酶测定试剂盒(时间分辨免疫荧光法)	国械注准 20163400539	中山大学达安基因股份有限公司
12	细胞角蛋白 19 片段测定试剂盒(时间分辨免疫荧光法)	国械注准 20163400531	中山大学达安基因股份有限公司

13	乙型肝炎病毒核酸测定试剂盒(PCR-荧光探针法)	国械注准 20163400142	中山大学达安基因股份有限公司
14	人类免疫缺陷病毒 1 型(HIV-1)核酸测定试剂盒(PCR-荧光探针法)	国械注准 20163400154	中山大学达安基因股份有限公司

2016 年实验室培养硕士研究生和博士研究生

1.2016 年毕业硕士研究生

序号	学生	毕业论文题目	导师
1	李坤饶	米托蒽醌的抗锥虫活性和作用机制的研究以及布氏锥虫 eIF3 复合物的组成鉴定与功能研究	郭学敏
2	吴姗姗	SOSTDC1 促进非小细胞肺癌细胞增殖的分子机制研究	黎孟枫
3	杨艳芳	PEDF 调控高游离脂肪酸血管内皮细胞转运及细胞内代谢的分子机制	蔡卫斌
4	黄昱昊	石墨烯量子点和 s-ACTH 片段对 Aβ 蛋白作用机制及纳米材料对中枢神经系统靶向载药的研究	顾怀宇
5	王意	TREM2/β-catenin 调控巨噬细胞焦亡在抗铜绿假单胞菌免疫中的作用研究	吴敏昊
6	徐峰	MDA5 及 RNA 解旋酶 A 参与细胞应激反应的作用研究	张萍
7	张雪飞	新型金属 β-内酰胺酶 IMP-45 的特征研究	田国宝
8	陆诗淼	环二核苷酸 cGAMP 治疗炎症性肠病及其作用机制研究	黄曦
9	杨宜英	IGRAs 试验在结核病诊断中的应用	黄曦
10	刘晋锋	Galectin-9/Tim-3 通路在疟疾相关急性肺损伤小鼠模型中的作用	吕芳丽
11	周丽娜	华支睾吸虫溶血磷脂酶在华支睾吸虫致肝纤维化作用机制的研究	余新炳
12	石梦辰	Csseverin 与肝/胆管癌细胞凋亡、迁移和侵袭过程的相关性研究	余新炳
13	肖思宇	Tim-3/Gal-9 对伯氏疟原虫感染小鼠肝脏 CD68+Kupffer 细胞的调节作用研究	吕芳丽
14	宋天章	抗华支睾吸虫亲环素 A 抗体对急慢性炎症损伤的保护作用	胡旭初
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附：2016 年重点实验室发表的部分论文摘要

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17β-Oestradiol enhances the expansion and activation of myeloid-derived suppressor cells via signal transducer and activator of transcription (STAT)−3 signalling in human pregnancy

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Summary

During a successful pregnancy, the maternal immune system plays a critical role in maintaining immunotolerance towards semi-allogeneic fetal antigens. Recent studies have indicated that myeloid-derived suppressor cells (MDSCs) are active players in establishing fetal–maternal tolerance; however, the underlying mechanism remains poorly understood. In this study, we observed a significant expansion of monocytic MDSCs (M-MDSCs) in the peripheral blood of pregnant women, which suppressed T cell responses in a reactive oxygen species-dependent manner and required cell–cell contact. The number of M-MDSCs correlated positively with serum oestrogen and progesterone levels. Administration of 17β-oestradiol, but not progesterone, enhanced both the expansion and suppressive activity of M-MDSCs through signal transducer and activator of transcription (STAT)-3. Pretreatment with STAT-3 inhibitor JSI-124 almost completely abrogated the effects of 17β-oestradiol on MDSCs. Collectively, these results demonstrate that 17β-oestradiol-induced STAT-3 signalling plays an important role in both the expansion and activation of MDSCs during human pregnancy, which may benefit the development of novel therapeutic strategies for prevention of immune-related miscarriage.

Keywords: 17β-oestradiol, human pregnancy, MDSC, STAT-3

Introduction

Tolerance towards fetal alloantigens is the major challenge for maternal immune system during the course of pregnancy. The maternal–fetal interface between the uterine mucosa and the embryonic tissues comprises distinct compartments that undergo dramatic changes during pregnancy, including implantation, placentation, fetal growth and parturition [1,2]. In mammals, implantation of an embryo in the mother's womb involves a series of steps leading to effective cross-talk between embryo and mother. Once the embryo is implanted, it suppresses the maternal immune response and prevents being rejected. Simultaneously, the mother's immune system will develop a reaction against graft-*versus*-host to favour immunological tolerance [3–5]. Although some types of immune cells were demonstrated to participate in the establishment of fetomaternal tolerance, the detailed mechanisms still remain to be understood [6–8].

Human trophoblasts are kept in direct contact with maternal tissues. The expression of polymorphic major histocompatibility complex molecules of maternal and paternal origin on the surface of trophoblasts allows them to interact functionally with surrounding immune cells [9]. Meanwhile, some types of immune cells such as natural killer cells, dendritic cells and regulatory T cells are either enriched in or excluded from the decidua, which ultimately affect the pregnancy [8,10–12]. Furthermore, when endometrial stromal cells differentiate, genes encoding the T cell-attracting chemokines CXCL9–12 and CCL5 become silenced epigenetically in decidual stromal cells [13], which limits T cell trafficking to the decidua and attenuates the immune

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Virus-Like Particles Produced in *Pichia Pastoris* Induce Protective Immune Responses Against Coxsackievirus A16 in Mice

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Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: Coxsackievirus A16 (CA16) is one of the main causative agents of hand, foot, and mouth disease (HFMD), and the development of a safe and effective vaccine has been a top priority among CA16 researchers.

Material/Methods: In this study, we developed a *Pichia pastoris* yeast system for secretory expression of the virus-like particles (VLPs) for CA16 by co-expression of the P1 and 3CD proteins of CA16. SDS-PAGE, Western blot, and transmission electron microscopy (TEM) were performed to identify the formation of VLPs. Immunogenicity and vaccine efficacy of the CA16 VLPs were assessed in BABL/c mouse models.

Results: Biochemical and biophysical analysis showed that the yeast-expressed CA16 VLPs were composed of VP0, VP1, and VP3 capsid subunit proteins, and present spherical particles with a diameter of 30 nm, similar to the parental infectious CA16 virus. Furthermore, CA16 VLPs elicited potent humoral and cellular immune responses, and VLPs-immunized sera conferred efficient protection to neonatal mice against lethal CA16 challenge.

Conclusions: Our results demonstrate that VLPs produced in *Pichia pastoris* represent a safe and effective vaccine strategy for CA16.

MeSH Keywords: Coxsackievirus Infections • Immunogenetics • Pichia • Vaccines, Virus-Like Particle

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viruses

MDPI

Review

Viral Evasion of Natural Killer Cell Activation

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Abstract: Natural killer (NK) cells play a key role in antiviral innate defenses because of their abilities to kill infected cells and secrete regulatory cytokines. Additionally, NK cells exhibit adaptive memory-like antigen-specific responses, which represent a novel antiviral NK cell defense mechanism. Viruses have evolved various strategies to evade the recognition and destruction by NK cells through the downregulation of the NK cell activating receptors. Here, we review the recent findings on viral evasion of NK cells via the impairment of NK cell-activating receptors and ligands, which provide new insights on the relationship between NK cells and viral actions during persistent viral infections.

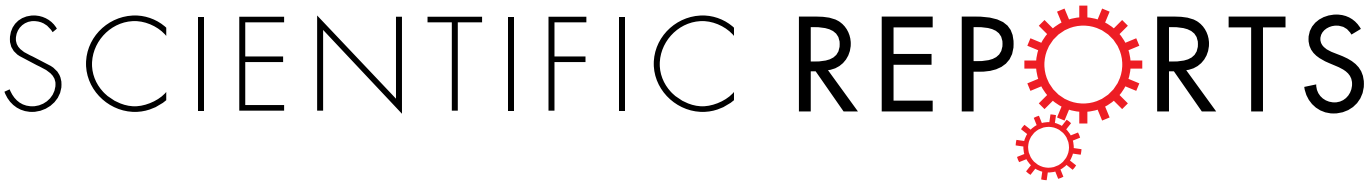
Keywords: natural killer cells; viral evasion; activating receptor; ligands; herpesvirus

1. Introduction

Natural killer (NK) cells serve as the first line of innate defense against viral infection, and they rapidly and directly kill infected cells in the absence of antigen presentation and recognition. In response to stimuli from diverse sources, including infections, cytokines, stresses and other immune cells, NK cells exert the following distinct functions: (i) secrete perforin and granzyme to directly kill target cells; (ii) release cytokines to regulate immune responses; and (iii) couple death-inducing receptors to target cells and induce apoptosis [1,2]. NK-deficient individuals are highly susceptible to a variety of viral infections, illustrating the key role of NK cells in the defense against viral infection [3]. However, viruses have evolved various strategies to evade the NK cell recognition and destruction during acute and persistent viral infections.

An array of activating or inhibitory receptors on the surface of NK cells recognize the ligands of target cells, and the relative expression of these receptors and the outcome of their signal cascades determines NK cell activation and cytotoxicity [4]. Numerous activating or inhibitory NK cell receptors have been identified in NK cells; the activating receptors recruit adaptors that contain the intracellular immunoreceptor tyrosine-based activating motif (ITAM), whereas the inhibitory receptors contain the immunoreceptor tyrosine-based inhibitory motifs (ITIM), consequently, they transduce activating or inhibitory signal cascades, respectively [5].

A cluster of inhibitory receptors specifically binds to major histocompatibility complex (MHC) class I molecules, such as the inhibitory Ly49s family members in mice, the killer-cell immunoglobulin-like receptors (KIR) in humans, and the heterodimeric CD94-NKG2A receptor in both species that recognizes non-classic MHC class I molecules. These molecules allow NK cells to be regulated by self-MHC recognition and restrain the NK cell hyperactivity [5]. Therefore, the NK cells preferentially kill the infected cells in which the surface expression of MHC molecules and the antigen presentation are inhibited by viruses [6].



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Using Baidu Search Index to Predict Dengue Outbreak in China

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This study identified the possible threshold to predict dengue fever (DF) outbreaks using Baidu Search Index (BSI). Time-series classification and regression tree models based on BSI were used to develop a predictive model for DF outbreak in Guangzhou and Zhongshan, China. In the regression tree models, the mean autochthonous DF incidence rate increased approximately 30-fold in Guangzhou when the weekly BSI for DF at the lagged moving average of 1–3 weeks was more than 382. When the weekly BSI for DF at the lagged moving average of 1–5 weeks was more than 91.8, there was approximately 9-fold increase of the mean autochthonous DF incidence rate in Zhongshan. In the classification tree models, the results showed that when the weekly BSI for DF at the lagged moving average of 1–3 weeks was more than 99.3, there was 89.28% chance of DF outbreak in Guangzhou, while, in Zhongshan, when the weekly BSI for DF at the lagged moving average of 1–5 weeks was more than 68.1, the chance of DF outbreak rose up to 100%. The study indicated that less cost internet-based surveillance systems can be the valuable complement to traditional DF surveillance in China.

Dengue fever (DF) is a major public health concern, particularly in the tropical and sub-tropical regions. The incidence of DF has increased 30-fold over the last five decades; between 1990 and 2013, dengue has been estimated to account for approximately ten thousand deaths per year[1,2]. As a fast spreading vector-borne infectious disease, DF is endemic to over 120 countries[3]. Recent estimates indicate that 390 million people suffer DF each year, including 96 million new cases[4]; this estimate is three times that of the DF burden estimated by WHO in 2009[5]. In China, DF cases have been reported in Guangdong, Hainan, Fujian, Yunnan, and Zhejiang every year, since the first DF outbreak in 1978. The burden of DF has increased with a number of large outbreaks occurring over the previous ten years; the most recent major outbreak resulted in over 40,000 cases in Guangdong province in 2014.

Prevention and control of DF primarily focuses on case surveillance, vector control, and DF vaccine initiatives. Although the first DF vaccine was registered in 2015 in Mexico[3], however, further testing of the vaccines efficacy need to be performed to allow its use in other countries. Traditional surveillance systems for DF are built on the basis of passive or sentinel site surveillance in the outpatient services or hospitals. These systems are limited by underreporting, delayed diagnosis and under-resourced laboratory services which limits case confirmation[6]. The development of real-time and accurate infectious disease surveillance remains a real challenge worldwide.

Digital surveillance systems that are built on internet search engines data can provide health authorities with important information regarding the emergence and spread of diseases in the community which can be used to complement traditional healthcare-based surveillance systems[7]. For example, Google Flu Trend (http://www.google.org/flutrends/) was used in the accurate real-time tracking of influenza outbreaks in some studies[8–10]. Moreover, the real-time detection and prediction using the internet-based surveillance systems have also been explored in some other diseases such as Ebola, malaria, and breast cancer[11–13]. One novel method for exploring

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SHORT COMMUNICATION

Up-regulated TLR2 and TLR4 expressions in liver and spleen during acute murine *T. gondii* infection

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Abstract Toll-like receptors (TLRs) play a central role in the pathogen clearance and pathological processes. The liver is an important innate immune organ, in which Kupffer cells and hepatocytes are important innate immune cells. However, the role of TLR2 and TLR4 in the liver caused by *Toxoplasma gondii* infection remains less clear. In this study, mice were infected with *T. gondii* RH strain and the grades of liver and spleen injuries were histopathologically evaluated. $TLR2^+$ and $TLR4^+$ cells in the livers and spleens were detected by immunohistochemistry, and their messenger RNA (mRNA) expressions were detected using quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR). The pathological severities in the livers and spleens were increased with time in *T. gondii*-infected mice. Compared with uninfected controls, obvious $TLR2^+$ and $TLR4^+$ cells were observed in the livers and spleens infected with *T. gondii* at 8 days post-infection, accompanied with significantly over-expressed mRNA levels of TLR2 and TLR4 in the livers and spleens after infection. Our data indicated that increased levels of TLR2 and TLR4 in the liver and spleen may play an important role during acute *T. gondii* infection.

Keywords *T. gondii* · Liver · TLR2 · TLR4 · Mice

Jialing Peng and Xiancan Lin contributed equally to this work.

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Introduction

Toxoplasma gondii is a ubiquitous intracellular protozoan parasite that infects about one third of the population for its extremely broad host and tissue specificity (Hill et al. 2005). After people ingest food or water contaminated with oocysts shed by cats or eat raw or undercooked meat carrying tissue cysts of infected animals, *T. gondii* disseminates throughout the body and then invades the tissues resulting in histopathological changes in the eye, brain, lung, liver, etc. (Alvarado-Esquivel et al. 2015). It is well known that the progression and severity of a disease depend on the immunological status of the host. In immunosuppressed host, cyst reactivation can cause severe or fatal encephalitis and/or disseminated toxoplasmosis (Dzierszinski et al. 2004). Protein antigens of *T. gondii* not only contain epitope structures for B cells, T cells , cytotoxic T lymphocytes, and NK cells to mediate immunological responses but also can contain structures that are unfavorable for protective immunity (Wang et al. 2016). Cases of toxoplasmic encephalitis, toxoplasmic hepatitis, and pulmonary toxoplasmosis have been reported among immunocompromised patients (Atilla et al. 2015; Desoubeaux et al. 2016; Mattie et al. 2016).

Toll-like receptors (TLRs) are a family of pattern-recognition receptors that play a critical role in the activation of innate immune system by recognizing pathogen-associated molecular patterns (PAMPs) (Akira et al. 2010). TLRs are expressed on Kupffer cells (KCs), dendritic cells (DCs), hepatic stellate cells, endothelial cells, and hepatocytes in the liver. TLR stimulation

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ORIGINAL PAPER

Upregulated Tim-3/galectin-9 expressions in acute lung injury in a murine malarial model

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Abstract Malaria is the most relevant parasitic disease worldwide, and severe malaria is characterized by cerebral edema, acute lung injury (ALI), and multiple organ dysfunctions; however, the mechanisms of lung damage need to be better clarified. In this study, we used Kunming outbred mice infected with *Plasmodium berghei* ANKA (*Pb*ANKA) to elucidate the profiles of T cell immunoglobulin and mucin domain-3 (Tim-3) and its ligand galecin-9 (Gal-9) in the development of ALI. Mice were injected intraperitoneally with 10^6 *Pb*ANKA-infected red blood cells. The lungs and mediastinal lymph nodes (MLNs) were harvested at days 5, 10, 15, and 20 post infections (p.i.). The grade of lung injury was histopathologically evaluated. Tim-3- and Gal-9-positive cells in the lungs and MLNs were stained by immunohistochemistry, and the messenger RNA (mRNA) expressions of Tim-3, Gal-9, and related cytokines were assessed using quantitative real-time polymerase chain reaction (qRT-PCR). Bronchoalveolar lavage fluid (BALF) analyses were performed from days 18 to 20 p.i. The results showed that the pathological severities in the lungs were increased with times and the total protein level in the BALFs was significantly elevated in *Pb*ANKA-infected mice. The numbers of Gal-9$^+$ and Tim-3$^+$ cells in the lungs were significantly increased, and the mRNA levels of both Gal-9 and Tim-3 in the lungs and MLNs were over-expressed in *Pb*ANKA-infected mice. In conclusion, our data suggested that Tim-3/Gal-9 may play a role in *Pb*ANKA-induced ALI.

Keywords *Plasmodium berghei* · Mice · Acute lung injury · Tim-3 · Galecin-9

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Introduction

Malaria is a common infection in the world. *Plasmodium* infection may result in severe malaria in patients infected with *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium knowlesi*, which can develop malaria-associated acute lung injury/acute respiratory distress syndrome (ALI/ARDS) and often results in morbidity and mortality. ALI or ARDS is with mortality rates of approximately 80 % (Taylor et al. 2012; White et al. 2013), accompanied by pulmonary edema (Taylor et al. 2006). Malaria-associated ALI/ARDS is thought to be due, in part, to increased alveolar permeability, parasite sequestration, and host immune response; however, the mechanisms behind it are largely unknown (Mohan et al. 2008).

T cell Ig and mucin domain-containing molecules (TIMs) are key regulators of immune responses (Rodriguez-Manzanet et al. 2009). Galectins are a family of highly conserved glycan-binding proteins that play an important role in the innate and adaptive immune responses (Rabinovich and Toscano 2009). Galectin-9 (Gal-9) down-regulates T helper (Th)1 and Th17 responses and is involved in the suppression mediated by CD4$^+$ CD25$^+$ T regulatory (Treg) cells, mainly through interaction with the Th1-specific cell surface molecule TIM-3 (Seki et al. 2008; Chou et al. 2009). The Gal-9/

Two distinct cytokinesis pathways drive trypanosome cell division initiation from opposite cell ends

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Cytokinesis in *Trypanosoma brucei*, an early branching protozoan, occurs along its longitudinal axis uni-directionally from the anterior tip of the new flagellum attachment zone filament toward the cell's posterior end. However, the underlying mechanisms remain elusive. Here we report that cytokinesis in *T. brucei* is regulated by a concerted action of Polo-like kinase, Aurora B kinase, and a trypanosome-specific protein CIF1. Phosphorylation of CIF1 by Polo-like kinase targets it to the anterior tip of the new flagellum attachment zone filament, where it subsequently recruits Aurora B kinase to initiate cytokinesis. Consistent with its role, CIF1 depletion inhibits cytokinesis initiation from the anterior end of the cell, but, surprisingly, triggers cytokinesis initiation from the posterior end of the cell, suggesting the activation of an alternative cytokinesis from the opposite cell end. Our results reveal the mechanistic roles of CIF1 and Polo-like kinase in cytokinesis initiation and elucidate the mechanism underlying the recruitment of Aurora B kinase to the cytokinesis initiation site at late anaphase. These findings also delineate a signaling cascade controlling cytokinesis initiation from the anterior end of the cell and uncover a backup cytokinesis that is initiated from the posterior end of the cell when the typical anterior-to-posterior cytokinesis is compromised.

cytokinesis | Polo-like kinase | Aurora B kinase | backup cytokinesis | *Trypanosoma brucei*

In eukaryotes, regulation of cytokinesis, the final step of cell division, involves a complex interplay of numerous proteins at the cytokinesis initiation site and the cleavage furrow. The mechanisms underlying cytokinesis in fungi and metazoa have been well understood, and the core regulatory pathways appear to be evolutionarily conserved (1). Along the cell division plane, which is defined by the position of the central spindle or the nucleus, animals and fungi assemble an actomyosin contractile ring, the cytokinesis apparatus that appeared about 1 billion years ago in the common ancestor of fungi, amoebas, and animals (2). In metazoa, the signaling pathway driving the transition from mitosis to cytokinesis involves two evolutionarily conserved protein kinases, the Polo-like kinase and the Aurora B kinase. Both kinases are concentrated on the central spindle and the midbody during late cell cycle stages and cooperate to recruit the centralspindlin complex to the central spindle and the midbody (3). Subsequently, the centralspindlin complex recruits Ect2, a guanine nucleotide exchange factor, to the midbody, which then recruits and activates the small GTPase RhoA at the midbody. Activation of RhoA further promotes the formation of the actomyosin contractile ring to drive cytokinesis (3).

Unlike many eukaryotic organisms that divide along the cell's short axis, the early branching protozoan *Trypanosoma brucei* undergoes cytokinesis along its longitudinal axis (4). The cell division plane in *T. brucei* is positioned by the newly assembled flagellum and its associated cytoskeletal structure termed the flagellum attachment zone (FAZ) filament (5, 6). Thus, cytokinesis is initiated from the anterior tip of the new FAZ filament, and cleavage furrow ingression occurs uni-directionally along the longitudinal axis toward the posterior end of the cell (4, 7) without forming an actomyosin contractile ring at the cleavage furrow (8).

As in fungi and metazoa, the Polo-like kinase (TbPLK in *T. brucei*) and the Aurora B kinase (TbAUK1 in *T. brucei*) are also required for cytokinesis in *T. brucei* (9, 10). TbPLK is concentrated in the flagellar basal body and the bilobe at late G1 phase, but from early S phase it is concentrated at the new FAZ tip and remains there until early anaphase (11). At the new FAZ tip, TbPLK is believed to promote cytokinesis initiation, but the underlying mechanism is unclear. TbAUK1 forms an unusual chromosomal passenger complex (CPC) with TbCPC1 and TbCPC2, and the complex displays a dynamic localization during the cell cycle. The complex is located in kinetochores from S phase to metaphase and on the central spindle during anaphase, but finally is degraded at the central spindle after late anaphase. However, starting from late anaphase, newly synthesized CPC proteins emerge at the new FAZ tip and then transfer to the cleavage furrow during cytokinesis (12). Localization of TbAUK1 to the new FAZ tip at late anaphase is crucial for cytokinesis initiation (13), but how it is recruited remains mysterious. The sequential recruitment of TbPLK and TbAUK1 to the new FAZ tip led us to hypothesize that an unknown factor is targeted by TbPLK to the new FAZ tip, which subsequently recruits TbAUK1 for cytokinesis initiation.

Here we report the identification of this factor, named CIF1, that links the TbPLK- and TbAUK1-signaling pathways. We also report the delineation of a cytokinesis regulatory pathway in

Significance

Cytokinesis occurs along a cell's short axis in many organisms, including bacteria, archaea, and eukaryotes. In many protozoa it occurs along the cell's longitudinal axis. The mechanism underlying this mode of cytokinesis is unknown. We delineate the signaling cascade that regulates cytokinesis along the longitudinal axis of *Trypanosoma brucei*, which is totally different from that in its human host. Additionally, we discover an alternative cytokinesis pathway that drives trypanosome cell division along the same division plane as the typical cytokinesis, but in an opposite direction. This alternative cytokinesis is activated only when the typical cytokinesis pathway is defective, suggesting that trypanosomes have evolved a backup cytokinesis mechanism to prevent the failure of cell division, thereby ensuring survival of this organism.

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The authors declare no conflict of interest.

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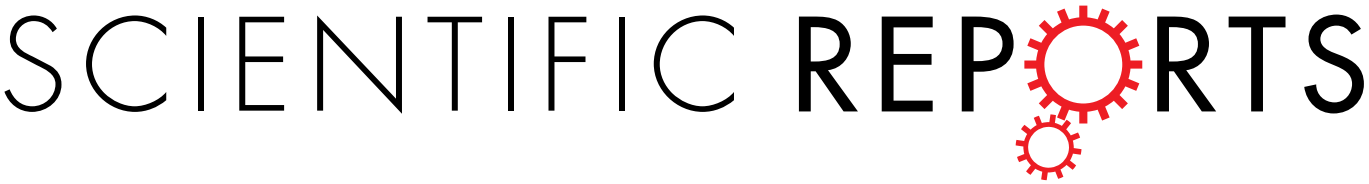

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TREM-2 promotes acquired cholesteatoma-induced bone destruction by modulating TLR4 signaling pathway and osteoclasts activation

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Triggering receptor expressed on myeloid cells (TREM) has been broadly studied in inflammatory disease. However, the expression and function of TREM-2 remain undiscovered in acquired cholesteatoma. The expression of TREM-2 was significantly higher in human acquired cholesteatoma than in normal skin from the external auditory canal, and its expression level was positively correlated with the severity of bone destruction. Furthermore, TREM-2 was mainly expressed on dendritic cells (DCs). In human acquired cholesteatoma, the expression of proinflammatory cytokines (IL-1β, TNF-α and IL-6) and matrix metalloproteinases (MMP-2, MMP-8 and MMP-9) were up-regulated, and their expression levels were positively correlated with TREM-2 expression. Osteoclasts were activated in human acquired cholesteatoma. In an animal model, TREM-2 was up-regulated in mice with experimentally acquired cholesteatoma. TREM-2 deficiency impaired the maturation of experimentally acquired cholesteatoma and protected against bone destruction induced by experimentally acquired cholesteatoma. Additional data showed that TREM-2 up-regulated IL-1β and IL-6 expression via TLR4 instead of the TLR2 signaling pathway and promoted MMP-2 and MMP-8 secretion and osteoclast activation in experimentally acquired cholesteatoma. Therefore, TREM-2 might enhance acquired cholesteatoma-induced bone destruction by amplifying the inflammatory response via TLR4 signaling pathways and promoting MMP secretion and osteoclast activation.

Human acquired cholesteatoma was identified more than 3 centuries ago and has a high morbidity rate; approximately 9 per 100,000 individuals are diagnosed annually around the world[1]. Characterized by constant keratinized epithelial proliferation[2] and temporal bone destruction[3], human acquired cholesteatoma can erode ossicles and temporal bone and destroy inner structures, such as vascular, neural and adjacent central nervous system structures[4]. This disease causes hearing loss, labyrinthitis, facial paralysis and even brain abscess[5]. Most otologists consider that acquired cholesteatoma-induced bone destruction is an extremely complicated process that involves many factors, such as mechanical pressure, inflammatory response, MMP expression, osteoclast activation and pH changes. Recent studies have demonstrated that the inflammatory response is the most important factor in inflammatory disease-induced bone destruction.

The bone destruction process can be divided into two stages. The first stage is bone matrix destruction, which has been proven to be the starting point of the entire bone destruction process and is mainly accomplished by

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TMEM2 inhibits hepatitis B virus infection in HepG2 and HepG2.2.15 cells by activating the JAK–STAT signaling pathway

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We have previously observed the downregulation of TMEM2 in the liver tissue of patients with chronic hepatitis B virus (HBV) infection and in HepG2.2.15 cells with HBV genomic DNA. In the present study, we investigated the role and mechanism of TMEM2 in HepG2 and HepG2.2.15 during HBV infection HepG2 and HepG2.2.15. HepG2 shTMEM2 cells with stable TMEM2 knockdown and HepG2 TMEM2 and HepG2.2.15 TMEM2 cells with stable TMEM2 overexpression were established using lentivirus vectors. We observed reduced expression of TMEM2 in HBV-infected liver tissues and HepG2.2.15 cells. HBsAg, HBcAg, HBV DNA, and HBV cccDNA levels were significantly increased in HepG2 shTMEM2 cells but decreased in HepG2 TMEM2 and HepG2.2.15 TMEM2 cells compared with naive HepG2 cells. On the basis of the western blotting results, the JAK–STAT signaling pathway was inhibited in HepG2 shTMEM2 cells but activated in HepG2 TMEM2 and HepG2.2.15 TMEM2 cells. In addition, reduced and increased expression of the antiviral proteins MxA and OAS1 was observed in TMEM2-silenced cells (HepG2 shTMEM2 cells) and TMEM2-overexpressing cells (HepG2 TMEM2 and HepG2.2.15 TMEM2 cells), respectively. The expression of Interferon regulatory factor 9 (IRF9) was not affected by TMEM2. However, we found that overexpression and knockdown of TMEM2, respectively, promoted and inhibited importation of IRF9 into nuclei. The luciferase reporter assay showed that IRF9 nuclear translocation affected interferon-stimulated response element activities. In addition, the inhibitory effects of TMEM2 on HBV infection in HepG2 shTMEM2 cells was significantly enhanced by pre-treatment with interferon but significantly inhibited in HepG2.2.15 TMEM2 cells by pre-treatment with JAK1 inhibitor. TMEM2 inhibits HBV infection in HepG2 and HepG2.2.15 by activating the JAK–STAT signaling pathway.
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Chronic hepatitis B virus (HBV) infection is a global public health challenge. It has been estimated that two billion people worldwide are infected with HBV[1] and that ~350 million people have chronic HBV infection, which is associated with cirrhosis, liver failure, and hepatocellular carcinoma. Up to one million deaths annually are caused by HBV-related diseases.[2] However, the mechanism by which HBV infects HepG2 and HepG2.2.15 cells is not fully understood. Our previous study investigating susceptibility to HBV revealed significant differences in the expression of the p.Ser1254Asn gene between healthy individuals and patients with HBV infection. Expression of the transmembrane protein TMEM2 encoded by p.Ser1254Asn in normal liver tissues and HepG2 cells was significantly higher than that in liver tissues of patients with chronic HBV infection and in HepG2.2.15 cells with HBV genomic DNA, respectively,[3] suggesting that TMEM2 has an important role in inhibiting HBV infection in HepG2 and HepG2.2.15 cells. TMEM2 belongs to the interferon-inducible transmembrane protein superfamily. The biological functions of TMEM2 remain largely unknown. It has been reported that other members of the interferon-inducible transmembrane protein superfamily exhibit interferon-mediated antiviral functions.[4,5] The JAK–STAT signaling pathway regulates cell growth, survival, and differentiation, and is involved in pathogen resistance. Interestingly, Li *et al.*[6] reported that inhibition of STAT1 methylation led to the resistance of HBV to interferon alpha treatments. On the basis of previous findings, we hypothesized that the JAK–STAT signaling pathway is involved in the anti-HBV activity of TMEM2. In the present study, we investigated the interactions between TMEM2 and the JAK–STAT signaling pathway during HBV infection in HepG2 and HepG2.2.15 cells. First, we performed gain- and loss-of-function assays in HepG2 and HepG2.2.15 cells by stable TMEM2 overexpression or silencing. Second, the expression of a number of key components of the JAK–STAT signaling pathway was evaluated, and the effects of the JAK–STAT signaling pathway on TMEM2-mediated anti-HBV activities were investigated.

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Abbreviations: HBV, hepatitis B virus; CHB, chronic hepatitis B; HBsAg, hepatitis B virus surface antigen; HBcAg, hepatitis B virus core antigen; MxA, myxovirus resistance protein 1; OAS1, 2', 5'-oligoadenylate synthetase 1; IFN, interferon; NTCP, sodium taurocholate cotransporting polypeptide

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PERSPECTIVE

The prevalence, origin, and prevention of six human coronaviruses

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CORONAVIRUSES

Coronaviruses (CoVs) are a large group of viruses found in many species of animals around the world, particularly bats and wild birds. CoVs result in various clinical manifestations ranging from asymptomatic respiratory, hepatic, and enteric diseases to neurological diseases. CoVs are classified under the family *Coronaviridae* in the order *Nidovirales* (Gonzalez et al., 2003), comprising an enveloped, positive-strand genome of approximately 26.4–31.7 kb in length, the largest genome of any RNA virus identified to date (Gorbalenya et al., 2006; Brian and Baric, 2005; Woo et al., 2010). Under electron microscopy, the virus has a characteristic crowned appearance (hence the name "corona").

Based on the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV), CoVs have been classified into four genera, including *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus*. Phylogenetically, *Alphacoronavirus* includes two subgroups, A and B; *Betacoronavirus* is divided into lineages A, B, C, and D (Adams et al., 2015); and *Gammacoronavirus* and *Deltacoronavirus* have not yet been classified into subgroups. Although a large number of CoV hosts have been identified, bats and birds are the ideal hosts for CoVs. *Alphacoronavirus* and *Betacoronavirus*are dominated by bat CoVs, while bird CoVs dominate *Gammacoronavirus* and *Deltacoronavirus* (Woo et al., 2012). To date, more than 50 CoVs have been discovered and sequenced (http://www.ictvonline.org/virusTaxonomy.asp; see phylogenetic analysis reviewed in (Woo et al., 2012)).

Historically, CoVs are common viruses that infect people of all different ages. In most cases, CoVs cause mild to moderate upper-respiratory illness with symptoms including runny nose, cough, sore throat, and fever (http://www.cdc.gov/coronavirus/about/). However, some CoVs can cause severe illness. In 2003, severe acute respiratory syndrome CoV (SARS-CoV) caused a severe global outbreak in humans, particularly in China, indicating that this virus may cause interspecies transmission and lead to epidemic disease. Thus, research on CoVs has greatly increased since 2003 owing to the high morbidity and mortality of SARS in humans. In 2012, the occurrence of Middle East Respiratory Syndrome CoV (MERS-CoV) was first reported in the Middle East and subsequently spread to other areas, such as South Korea and Hong Kong. The common clinical symptoms of SARS and MERS include persistent fever, cough, chills, dyspnea, and headache. Additionally, these two HCoVs are both characterized by rapidly progressive pneumonia. Patients who contract SARS or MERS often die of chronic diseases, such as cardiovascular disease, respiratory disease, and diabetes. Moreover, CoVs have attracted great attention owing to the recent human-to-human transmission of MERS-CoV in South Korea, with a fatality rate four times higher than that of SARS-CoV (Durai et al., 2015). Many factors have blocked the prevention and control of these new epidemic diseases, including its rapid worldwide distribution, extensive genetic diversity, high rates of mutation and recombination, and lack of an effective vaccine or methods for treatment and prevention.

PREVALENCE OF HCOVS

Only two HCoVs, i.e., HCoV-229E and HCoV-OC43, had complete ge-

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Parasites & Vectors

RESEARCH Open Access

The immunological characteristics and probiotic function of recombinant *Bacillus subtilis* spore expressing *Clonorchis sinensis* cysteine protease

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Abstract

Background: Clonorchiasis, a food-borne zoonosis, is caused by *Clonorchis sinensis*. The intestinal tract and bile ducts are crucial places for *C. sinensis* metacercariae to develop into adult worms. The endospore of *Bacillus subtilis* is an ideal oral immunization vehicle for delivery of heterologous antigens to intestine. Cysteine protease of *C. sinensis* (*Cs*CP) is an endogenous key component in the excystment of metacercariae and other physiological or pathological processes.

Methods: We constructed a fusion gene of CotC (a coat protein)-*Cs*CP and obtained *B. subtilis* spores with recombinant plasmid of pEB03-CotC-*Cs*CP (*B.s*-CotC-*Cs*CP). CotC-*Cs*CP expressed on spores' surface was detected by Western blotting and immunofluorescence. Immunological characteristics of recombinant spore coat protein were evaluated in a mouse model. The levels of *Cs*CP-specific antibodies were detected by ELISA. Effects of recombinant spores on mouse intestine were evaluated by histological staining. The activities of biochemical enzymes in serum were assayed by microplate. Liver sections of infected mice were evaluated by Ishak score after Masson's trichrome.

Results: The *B.s*-CotC-*Cs*CP spores displayed *Cs*CP on their coat. Specific IgG and isotypes were significantly induced by coat proteins of *B.s*-CotC-*Cs*CP spores after subcutaneous immunization. IgA levels in intestinal mucus and bile of *B.s*-CotC-*Cs*CP orally treated mice significantly increased. Additionally, more IgA-secreting cells were observed in enteraden and *lamina propria* regions of the mouse jejunum, and an increased amount of acidic mucins in intestines were also observed. There were no significant differences in enzyme levels of serum among groups. No inflammatory injury was observed in the intestinal tissues of each group. The degree of liver fibrosis was significantly reduced after oral immunization with *B.s*-CotC-*Cs*CP spores.

Conclusions: *Bacillus subtilis* spores maintained the original excellent immunogenicity of *Cs*CP expressed on their surface. Both local and systemic specific immune responses were elicited by oral administration of *B.s*-CotC-*Cs*CP spores. The spores effectively promoted intestinal health by inducing secretion of acidic mucins, with no other side effects to the liver or intestine. Oral administration of spores expressing *Cs*CP could provide effective protection against *C. sinensis*. This study may be a cornerstone for development of antiparasitic agents or vaccines against clonorchiasis based on *B. subtilis* spore expressing *Cs*CP on the surface.

Keywords: *Clonorchis sinensis*, *Bacillus subtilis* spore, Cysteine protease, Oral immunization, Immunological characteristics

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OPEN

The Distribution of Human Stem Cell–like Memory T Cell in Lung Cancer

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Summary: Human stem cell–like memory T (Tscm) cells are long-lived, self-renewing memory lymphocytes that can differentiate into effector cells and mediate strong antitumour response in murine model. The distribution and function of Tscm cells in human lung cancer remain unknown. In this study, we investigated the properties of human Tscm cells in the blood and lymph node of non–small cell lung cancer (NSCLC) patients. There were more $CD4^+$ Tscm cells in blood from NSCLC patients than from healthy donors, fewer $CD4^+$ and $CD8^+$ TSCM cells in blood than in lymph node from NSCLC patients. To further analyze their properties, we stimulated peripheral blood mononuclear cells from NSCLC patients by mitogens to examine cytokine production. Our data suggest that both CD4 and CD8 Tscm cells in blood produced interferon-γ significantly increased in NSCLC patients compare with healthy subjects. In addition, fewer Tscm cells produced interferon-γ in lymph node than in blood from NSCLC patients. Our results strongly suggest that the distribution and function of CD4 Tscm cells in NSCLC patients is upregulated. Understanding of the properties of stem-like memory T cells will supply a good rationale for designing the new adoptive immunotherapy in cancer.

Key Words: stem cell–like memory T cell, lung cancer, memory T cell

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Long-lived memory lymphocytes are hallmark features of adaptive immune systems. Naive T cells are activated in response to pathogens and tumors. Most of the activated T cells die of apoptosis after undergoing stimulation and clonal expansion, whereas only a minority of activated T cells become memory T cells. These memory T cells provide rapid and strong protection against recurrent pathogens. The heterogeneity of memory T cells with respect to phenotypic markers, effector function, and tissue-homing capabilities can be categorized into 2 main populations: effector memory T cell (Tem) and central memory T cells (Tcm). Tcm cell preferentially reside in secondary lymphoid organs and express CCR7 and CD62L; whereas Tem reside in extra lymphoid sites but do not express CCR7. Tcm and Tem cells have immediate effector function against polyclonal or antigen stimulation.[1–4]

Generation and maintenance of the longevity of memory T cells require stem cell–like capacity to self-renew and differentiate into the subsets of memory T cells.[5] A new population of memory T cells with enhanced stem cell–like property was recently identified in mouse and humans.[6–9] Stem cell–like memory T (Tscm) cells exhibit analogous effector function to memory T cells, but they also have increased proliferation, self-renewal, and strong antitumour function compared with conventional memory T cells in murine model of melanoma.[10] One group reported that human memory stem cells occur naturally in peripheral blood mononuclear cell (PBMCs). Researchers also observed that $CD8^+$ Tscm cells exhibit a strong capacity to reject tumor growth both in murine tumor and melanoma model in human mice.[8,10] Tscm cells share both naive T cell and memory T cell characteristic phenotypes. Similar to naive T cells, Tscm cells express molecular marker CD45RA, CD62L, CCR7, stem cell–like associate marker CD127 and memory-associated markers CD27, CD28, CD95, and CD122. The CD95 signalling complex induces proapoptosis, and is preferentially expressed by $CD45RA^-CD45RO^+$ T cells but not $CD45RA^+$ $CD45RO^-$ T cells in adults.[11] CD122 [interleukin (IL)-2R β, the common β chain of the IL-2 and IL-15 receptors] can undergo homeostatic proliferation in response to IL-15 and IL-7. CD122 may be a relevant factor in controlling the development, programming, and survival of memory T cells.[12–14] After being treated with the WNT pathway activator TWS119, the expression of CD95 and CD122 was upregulated in Tscm cells, CD95 and CD122 might be considered as a marker of Tscm cells. Current studies demonstrated that antigen-specific memory T cells are a major target cell type of adoptive cell transfer in tumor immunotherapy and vaccine design,[15–20] $CD4^+$ helper T cells may have a critical function in improving cancer immunotherapies.[21–23] The antitumour responses of $CD8^+$ Tscm cells have been discovered in animal model,[10] but the compartmentalization and function of $CD4^+$ Tscm, $CD8^+$ Tscm in human non–small cell lung cancer (NSCLC) patient still remains unclear.

In this study, we analyze the properties of human Tscm cells and its subsets in PBMCs and lymph node from healthy donors (HDs) and NSCLC patient. The number of $CD4^+$ Tscm cells in blood of NSCLC patients was found to be higher compared with HDs. $CD4^+$ Tscm and $CD8^+$ Tscm cells were low in blood than in lymph nodes of NSCLC patients. The further functional analysis shows that both $CD4^+$ Tscm and $CD8^+$ Tscm cells in blood produced interferon (IFN)-γ in significantly increased

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OPEN

The Characteristics of Naive-like T Cells in Tumor-infiltrating Lymphocytes From Human Lung Cancer

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Summary: Adoptive cell therapy using autologous tumor-infiltrating lymphocytes (TILs) or genetically modified lymphocytes from TILs is a new effective approach, but the application of TIL immunotherapy is still limited in many solid tumors. Knowledge of the classification and function of TILs is important to develop personalized immunotherapy with TILs in non–small lung cancer (NSCLC). In this study, we show the characteristics of T-cell subsets in TILs isolated from NSCLC. $CD3^+$ $CD8^+$ $CD45RA^+$ T cells outnumbered $CD3^+$ $CD4^+$ $CD45RA^+$ T cells in $CD45RA^+$ TILs, but it was the opposite in $CD45RO^+$ TILs. Effector memory $CD4^+$ T cells predominated in $CD4^+$ TILs; about 10% of the stem cell-like memory T cells (Tscm) were detected in TILs. To further analyze their functions, we stimulated TILs from NSCLC patients by mitogens to examine cytokine production. Our data demonstrated that naive-phenotype T cells in TILs secret IFN-γ in abundance; TNF-α-producing T cells were significantly increased in TILs; there were more IL-17-expressing $CD4^+$ Tscm cells than other subtypes of $CD4^+$ T cells in TILs. Our findings indicate that the $CD4^+/CD8^+$ naive-phenotype T cells and Tscm cells in TILs from lung cancer exhibit distinct composition and strong cytokine production. Attributes of Tscm cells from a naive-like T-cell population in TILs are the promising cell type for adoptive cell therapy in human lung cancer.

Key Words: T cell, tumor-infiltrating lymphocytes, lung cancer

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Tumor-infiltrating lymphocytes (TILs) were first described in 1863 by Robert Virchow and were found in proximity of the tumor.[1] TILs are identified as a heterogenous cell group, include effector T cells, T regulatory cells, natural killer cells, macrophages, dendritic cells, myeloid-derived suppressor cells, and other immune cell types.[1] The major populations of TILs that grow from tumors are $CD8^+$ T cells and $CD4^+$ T cells in vitro culture.[2] The frequency of TILs was correlated with a better prognosis in patients with different types of tumors,[3–5] including non–small lung cancer (NSCLC).[6,7] The subtypes of TILs in NSCLC have been reported with different prognostic effect. Mori et al's[8] report indicates that the number of $CD8^+$ TILs in NSCLC is not with a favorable prognosis; $CD4^+$ T cells, not $CD8^+$ T cells, in NSCLC cancer nests are associated with a favorable prognosis.[9] Hiraoka et al's[10] study demonstrated that the infiltration of $CD8^+$ and $CD4^+$ TIL cells are a favorable prognostic factor in NSCLC. In 2010, another report shows that a high frequency of $CD8^+$ TILs in NSCLC tissues is correlated with a favorable prognosis.[11] In light of recent studies of immunotherapy, adoptive cell therapy (ACT) immunotherapy with autologous TILs with the memory phenotype yields drastic regression of malignant melanoma,[12–15] whereas transferring terminal differentiation of TILs have poor antitumor immunity and short-term persistence.[12,16] In other human advanced cancer (such as NSCLC), the efficacy of TIL therapy is uncertain. To further improve the antitumor effect and therapeutic potential of ACT with TILs in NSCLC, it is necessary to identify the composition and function of T cells in TILs.

According to the surface marker, the function and proliferation capacity, T cells can be categorized into naive T cells (Tn), effector T cells (Teff), and memory T cells (Tm); memory T cells include central memory T cells (Tcm), effector memory T cells (Tem), stem cell-like memory T cells (Tscm), and tissue-resident memory T cells (Trm).[17,18] The distribution and function of Tscm cells in human lung cancer in the peripheral blood and lymph nodes have been studied.[19] In this study, we investigated the characteristics of T cells in TILs from NSCLC. We found that $CD3^+$ $CD8^+$ $CD45RA^+$ T cells outnumbered $CD3^+$ $CD4^+$ $CD45RA^+$ T cells, whereas $CD3^+$ $CD4^+$ $CD45RO^+$ T cells outnumbered $CD3^+$ $CD8^+$ $CD45RO^+$ T cells. $CD4^+$ Tem cells predominated in $CD4^+$ TILs, the proportion of $CD8^+$ Teff cells was higher than that of $CD4^+$ Teff cells. About 12% of $CD4^+$ Tscm and 10% of $CD8^+$ Tscm were detected in TILs. To further analyze the function of T-cell subsets in TILs, we stimulated TILs from NSCLC patients with mitogens to examine cytokine production. Our data demonstrate that naive-phenotype T cells in TILs secret interferon-γ (IFN-γ) in abundance; tumor necrosis factor (TNF)-α-producing T cells were significantly increased, but fewer $CD8^+$ Tscm cells produced TNF-α than $CD4^+$ Tscm cells in TILs; there were more interleukin-17 (IL-17)-expressing $CD4^+$ Tscm cells than other subtypes of $CD4^+$ T cells in TILs. An accurate understanding of the subsets of T cells in TILs from NSCLC is critical for the prognosis and personalized medicine with TILs immunotherapy.

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Research Paper

TACC3 promotes colorectal cancer tumourigenesis and correlates with poor prognosis

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ABSTRACT

Colorectal carcinoma (CRC) is a malignant epithelial tumour with tremendous invasion and metastatic capacity. Transforming acidic coiled-coil protein-3 (TACC3), a frequently aberrantly expressed oncogene, is an important biomarker in various human cancers. Our study aimed to investigate the expression and function of TACC3 in human CRC. We found that TACC3 was over-expressed at both the mRNA and protein levels in CRC cells and in biopsies of CRC tissues compared with normal controls as determined by qRT-PCR, western blot and immunohistochemical (IHC) staining assays. IHC staining of samples from 161 patients with CRC also revealed that TACC3 expression was significantly correlated with clinical stage ($P = 0.045$), T classification ($P = 0.029$) and M classification ($P = 0.020$). Multivariate analysis indicated that high TACC3 expression was an independent prognostic marker for CRC. Patients who had high TACC3 expression had significantly poorer overall survival (OS, $P = 0.023$) and disease-free survival (DFS, $P = 0.019$) compared to patients who had low TACC3 expression. Furthermore, TACC3 knockdown attenuated CRC cell proliferation, colony formation capability, migration and invasion capability, and tumourigenesis in nude mice; these properties were measured using a real-time cell analyser (RTCA), clonogenicity analysis, and transwell and xenograft assays, respectively. These data indicate that TACC3 promotes CRC progression and could be an independent prognostic factor and a potential therapeutic target for CRC.

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Survival advantage depends on cecal volume rather than cecal length in a mouse model of cecal ligation and puncture

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ABSTRACT

Background: Cecal ligation and puncture (CLP) is the most commonly used model to simulate human polymicrobial sepsis. However, the severity of CLP is difficult to be standardized across different laboratories. The aim of the present study was to evaluate the influence of ligated cecal volume and length on mortality in mouse CLP model.

Methods: Cecal length and volume were measured from 120 Kunming mice subjected to CLP or sham operation. According to cecal volume, mice were divided into three groups, volume$_{0.0\sim0.2}$ (0.0 cm^3-0.2 cm^3), volume$_{0.2\sim0.4}$ (0.2 cm^3-0.4 cm^3), and volume$_{>0.4}$ (larger than 0.4 cm^3). The contents of cytokines, including interleukin-1β, interleukin-6, and TNF-α, were measured at 3 h after surgery. The blood bacterial load and oxidative stress indicators (including malondialdehyde and superoxide dismutase) were measured at 12 h after surgery.

Results: There was no significant difference on 72-h survival rate between the mice with cecum longer than 2 cm and shorter than 2 cm. Compared to the other volume groups, volume$_{>0.4}$ group showed significantly increased blood bacterial load, malondialdehyde levels in lung and liver, and pro-inflammatory cytokines in serum. Surprisingly, the survival rate in volume$_{>0.4}$ (0%) group showed significant difference from those of volume$_{0.0\sim0.2}$ group (40%) and volume$_{0.2\sim0.4}$ group (40%).

Conclusions: The mice in volume$_{>0.4}$ group have much serious inflammatory reaction and are easier to die. As the proportion of volume$_{>0.4}$ mice is near 20%, it can have large influence on most of the related studies using this CLP model.

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OPEN

RESEARCH ARTICLE

Suppressed expression of miR-378 targeting *gzmb* in NK cells is required to control dengue virus infection

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Dengue virus (DENV) remains a major public health threat because no vaccine or drugs are available for the prevention and treatment of DENV infection, and the immunopathogenesis mechanisms of DENV infection are not fully understood. Cytotoxic molecules, such as granzyme B (GrzB), may be necessary to control viral infections. However, the exact role of GrzB during DENV infection and the mechanisms regulating GrzB expression during DENV infection are not clear. This study found that miR-27a*, miR-30e, and miR-378 were down-regulated in DENV-infected patients, and DENV infection in humans induced a significant up-regulation of GrzB in natural killer (NK) cells and $CD8^+$ T cells. Further investigation indicated that NK cells, but not $CD8^+$ T cells, were the major sources of GrzB, and miR-378, but not miR-27a* or miR-30e, suppressed GrzB expression in NK cells. Notably, we found that overexpression of miR-378 using a miR-378 agomir in DENV-infected mice inhibited GrzB expression and promoted DENV replication. These results suggest the critical importance of miR-378 in the regulation of GrzB expression and a protective role for GrzB in controlling DENV replication *in vivo*. Therefore, this study provides a new insight into the immunopathogenesis mechanism of DENV infection and a biological basis for the development of new therapeutic strategies to control DENV infection.
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Keywords: dengue virus; granzyme B; miRNA-378; NK cells

INTRODUCTION

Dengue virus (DENV) is a positive-polarity, single-stranded RNA virus in the mosquito-borne flavivirus family. DENV has four infectious serotypes: DENV-1, -2, -3, and -4. DENV infection results in clinical diseases ranging from an asymptomatic, acute self-limiting febrile illness, such as dengue fever, to a much more severe life-threatening form of dengue infection that is characterized by high fever, large-scale hemorrhage, plasma leakage, and multiple organ failure, such as dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS).[1,2] More than 50 million people annually contract DENV, which leads to approximately 500 000 hospitalizations and 25 000 deaths, particularly children.[3] Therefore, DENV remains a serious public health threat in tropical and subtropical areas.[3] Unfortunately, there are no drugs or vaccines that target DENV. Elucidation of the immunopathogenesis mechanisms of DENV infection is critically important for the development of anti-DENV drugs and vaccines.

DENV infection induces massive immune activation and the production of high amounts of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), which may contribute to the immunopathogenesis of severe DENV infection, such as DHF/DSS.[4–6] Cellular cytotoxic molecules, such as perforin and granzymes, may also contribute to the progression of DENV infection and the development of massive vascular leaks that lead to DHF/DSS,[7,8] particularly in secondary dengue infection with heterologous serotypes.

Natural killer (NK) cells, $CD8^+$ T cells, and a few $CD4^+$ T cells are the major sources of perforin and GrzB expression.[9,10] NK cells and DENV-specific $CD8^+$ T cells are likely activated at a very early stage during an acute DENV infection.[11] These cells produce cytokines, cytotoxic molecules, and adhesion molecules[11–14] and demonstrate potent cytotoxic activity,[14] which may promote the development of an efficient adaptive immune response by $CD4^+$ T cells.[15] NK cells and DENV-specific $CD8^+$

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ORIGINAL PAPER

Spleen atrophy related immune system changes attributed to infection of *Angiostrongylus cantonensis* in mouse model

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Abstract The spleen is one of the most important peripheral immune organs, which is frequently affected in infectious diseases. Infectious diseases can induce splenic alterations including splenic atrophy and functional alteration, while splenic atrophy may in turn interferes with recovery of infectious diseases. Angiostrongyliasis is an infectious disease by *Angiostrongylus cantonensis* (*A. cantonensis*), which invade non-permissive hosts, such as humans and mice, to cause severe damage to the central nervous system (CNS) and acute inflammatory response. *A. cantonensis* infection-induced CNS injury has been confirmed to be due to profound immunopathology derived from peripheral immune components. However, the mechanism of immunopathology remains largely unknown. Here, we found that *A. cantonensis* invaded non-permissive hosts such as mice in the brain, but not in the other peripheral organs. However, this infection induced severe spleen atrophy. We further recognized that this atrophy is associated with a decrease of total splenocyte number and disruption of splenic structure due to reduced proliferation and increased apoptotosis. These also resulted in deterioration of T cell profile in the periphery with a low CD4/CD8 ratio and B/T cell ratio, and increased ratio of $CD4^+CD25^+Foxp3^+$ Treg, $CD8^+CD28^-$ T, and $CD38^+$T lymphocyte of spleen. Albendazole treatment can alleviate spleen atrophy and set T cell immune reconstitution in some extend. Our data showed that *A. cantonensis* infection can cause splenic atrophy. These results are suggested to put more emphasis to improve the function of immune system. Meanwhile, infection and treatment model will be useful to evaluate new therapeutic approaches which can prevent or reverse immunosuppression and infectious complications.

Keywords *Angiostrongylus cantonensis* · CNS · Spleen atrophy · Treg cells · CD28 · CD38

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RESEARCH Open Access

SOSTDC1 is down-regulated in non-small cell lung cancer and contributes to cancer cell proliferation

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Abstract

Background: Non-small cell lung cancer (NSCLC) is the most commonly diagnosed and fatal cancer worldwide. Sclerostin domain containing protein 1 (SOSTDC1) has been found to be tumor-suppressive in several types of cancers. However, the expression level and biological functions of SOSTDC1 in NSCLC remain unknown. Our current study aimed to identify the biological significance of SOSTDC1 in NSCLC.

Results: We found that SOSTDC1 was significantly down-regulated in NSCLC. Moreover, patients with higher expression of SOSTDC1 had a significant better prognosis than those with lower SOSTDC1 expression. Ectopic expression of SOSTDC1 in NSCLC cell lines A549 and NCI-H520 could inhibit proliferation as shown by MTT, colony formation, soft agar and EdU incorporation assays in vitro. Furthermore, A549 cells stably expressing ectopic SOSTDC1 grew more slowly and formed smaller tumors than vector-control cells in vivo. Mechanistic studies demonstrated that SOSTDC1 over-expression led to increased p21Cip and p27Kip levels, thereby decreasing Rb phosphorylation status and E2F transcription activity.

Conclusions: SOSTDC1 is down-regulated in NSCLC, and its expression level is indicative of clinical outcome of patients with the disease. SOSTDC1 might represent a tumor suppressor through inhibiting the proliferation of NSCLC cells by regulating p21Cip and p27Kip, which in turn affects Rb-E2F signaling.

Keywords: SOSTDC1, Non-small cell lung cancer, Proliferation, p21Cip, p27Kip

Background

Lung cancer is one of the most commonly diagnosed cancer types worldwide and the leading cause of cancer-related death [1, 2]. Non-small cell lung cancer (NSCLC), which includes squamous cell carcinoma (SCC), adenocarcinoma (AD), large cell carcinoma (LCC), and other less frequently diagnosed histological types, accounts for about 80 % of lung cancer cases [3]. While various therapeutic approaches, including surgical resection, chemo- and radio-therapies, have been applied in the management of NSCLC, the overall 5-year survival rate of NSCLC patients still remains at only 15 % [4]. Better understanding of the genetic events and key molecules involved in NSCLC development and progression is needed for developing effective therapeutic strategies against the disease.

Sclerostin domain-containing protein 1 (SOSTDC1), an important regulator of cell signaling, has been found to contribute to several physiological and pathological processes [5, 6]. Accumulating evidence has revealed that SOSTDC1 might act as a tumor suppressor in many cancers. In wilms tumor, SOSTDC1 is lost as a result of a 7p21 homozygous deletion, which led to accelerate angiogenesis and activation of Wnt signaling [7]. The expression of SOSTDC1 is down-regulated in gastric cancer, and ectopic over-expression of SOSTDC1 in gastric

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ORIGINAL PAPER

Soluble antigen derived from *IV* larva of *Angiostrongylus cantonensis* promotes chitinase-like protein 3 (*Chil3*) expression induced by interleukin-13

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Abstract Angiostrongyliasis caused by *Angiostrongylus cantonensis (A. cantonensis)* is an emerging food-borne parasitic disease, which refers basically to eosinophilic meningitis. Chitinase-like protein 3 (*Chil3*), a member of chitinase-like protein family which has chemotactic activity for eosinophils, is reported to be highly upregulated in brain of mouse infected with *A. cantonensis*. The mechanisms of high expression of *Chil3* and the association between *A. cantonensis* and *Chil3* are rarely reported. In order to understand the mechanism of high expression of *Chil3* in *A. cantonensis*-infected mouse, we measured the level of *Chil3* in RAW 264.7 and BV2 cell lines stimulated with soluble antigen of *A. cantonensis* by qPCR and ELISA. To explore the role of *Chil3* in inflammation caused by *A. cantonensis*, we extracted and cultured brain mononuclear cells (BMNCs) and detected the eosinophil chemotactic activity of *Chil3* using transwell assay and flow cytometer. Furthermore, we treated the infected mice by injection with *rmChil3* and then counted the number of larvae in brains of infected mice and treated mice to examine the association between the worm and *Chil3*. Our results showed the soluble antigen from *A. cantonensis* could promote the *Chil3* expression in macrophage and microglial cell lines induced by interleukin-13. In conclusion, we supposed that high expression of *Chil3* enhanced by soluble antigens from *A. cantonensis* might be the reason of serious eosinophil infiltration in mouse brain after *A. cantonensis* infection.

Keywords *Angiostrongylus cantonensis* · *Chil3* · Eosinophil · Interleukin-13 · Antigen

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Introduction

Angiostrongylisis is one of the food-borne parasitic diseases and caused by the rat lung worm, *Angiostrongylus cantonensis* (*A. cantonensis*). The parasite was first discovered in China by Xintao Chen in 1935 (Chen 1935) and was neglected until 1944 when the first angiostrongylisis patient was found in Taiwan (Rosen et al. 1961). Approximately 2900 cases of human angiostongyliasis had been reported from more than 30 countries, with no doubt that many more cases were unreported because of lacking awareness of *A. cantonensis* (Wang et al. 2012). Currently, angiostrongylisis is regarded as a public health problem. Rat is known as definitive host. However, human and mouse are accidental hosts (non-definitive hosts). Humans become infected by eating raw or improperly cooked freshwater snails, paratenic hosts such as frogs and fish, or vegetables polluted by the third-stage larvae (L3) of *A. cantonensis* (Eamsobhana et al. 2009; Lai et al. 2007; Sinawat et al. 2008; Wallace and Rosen 1966). L3 reach the central nervous system (CNS) in both definitive and non-definitive hosts and then develop into young adults. In rat, the young adults return to lung and become sexually mature.

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REVIEW

Should we abandon quinine plus antibiotic for treating uncomplicated *falciparum* malaria? A systematic review and meta-analysis of randomized controlled trials

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Abstract In this study, we compared the efficacies and adverse effects of quinine plus antibiotics and other anti-malaria drugs on treating uncomplicated *falciparum* malaria. By systematically searching the major databases PubMed, Embase, and the Cochrane Library, 14 randomized controlled trials (RCTs) including 1996 cases were identified. Then, we performed a systematic review and cumulative meta-analysis on these data. The primary outcome of these treatments was parasite failure at day 28. There was no significant difference between quinine plus antibiotic therapy (QACT) and artemisinin-based therapies (odds ratio (OR) 0.69, 95 % confidence interval (CI) 0.28 to 1.71) or non-artemisinin-based therapies except quinine monotherapy and chloroquine monotherapy (OR 0.56, 95 % CI 0.18 to 1.74). The secondary outcome was the adverse effects within 28 days, including nausea, dizziness, vomiting, diarrhea, abdominal pain, headache, and tinnitus. QACT significantly increased the risk of tinnitus compared with artemisinin-based therapies (OR 111.65, 95 % CI 12.63 to 986.87) and non-artemisinin-based therapies (OR 48.16, 95 % CI 16.23 to 142.92). Vomiting was more frequently reported in QACT compared with non-artemisinin-based therapies (OR 2.02, 95 % CI 1.14 to 3.56). This meta-analysis suggests that almost all regimens have equivalent treatment effect at the 28th day. However, the patients with QACT had a higher chance to suffer from vomiting and tinnitus. Therefore, QACT does not have significant advantage on treating uncomplicated *falciparum* malaria.

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ORIGINAL PAPER

Recombinant Sj16 from *Schistosoma japonicum* contains a functional N-terminal nuclear localization signal necessary for nuclear translocation in dendritic cells and interleukin-10 production

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Abstract Sj16 is a *Schistosoma japonicum*-derived protein (16 kDa in molecular weight) that has been identified as an immune modulation molecule, but the mechanisms of modulation of immune responses are not known. In this report, we aimed to investigate the host immune regulation mechanism by recombinant Sj16 (rSj16) and thus illuminate the molecular mechanism of immune evasion by *S. japonicum*. The effect of rSj16 and rSj16 mutants on the biology of dendritic cells (DCs) was assessed by examining DC maturation, cytokine production, and expression of surface markers by flow cytometry and enzyme-linked immunosorbent assay. We found that rSj16 significantly stimulated interleukin (IL)-10 production and inhibited LPS-induced bone marrow-derived dendrite cell (BMDC) maturation in a dose-dependent manner. By using antibody neutralization experiments and IL-10-deficient (knockout) mice, we confirmed that the inhibitory effect of rSj16 on LPS-induced BMDCs is due to its induction of IL-10 production. To understand how rSj16 induces the production of IL-10, we analyzed the protein sequence and revealed two potential nuclear localization signals (NLS) in Sj16. The N-terminal NLS (NLS1) is both necessary and sufficient for translocation of rSj16 to the nucleus of BMDCs and is important for subsequent induction of IL-10 production and the inhibition of BMDC maturation by rSj16. The results of our study concluded that the ability of rSj16 to inhibit DC functions is IL-10 dependent which is operated by IL-10R signal pathway. This study also confirmed that NLS is an important domain associated with increased production of IL-10. Our findings will extend the current understanding on host-schistosome relationship and provide insight about bottleneck of parasitic control.

Keywords Schistosoma japonicum · rSj16 · IL-10 · Nuclear localization signal

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Introduction

Schistosomiasis, which is caused by infection with parasitic flatworms, remains a public health burden in many developing countries in the tropics and subtropics (Utzinger et al. 2005). Effective control and treatment rely on chemotherapy by using praziquantel, which remains the only drug in the mainstay of current medical treatment (Harder 2002). However, praziquantel does not prevent reinfection, and resistant strains may develop in the future. Development of a vaccine is ultimately required, and, as yet, no effective vaccines are available (Mackinnon et al. 2008). The most important reason for the lack of a vaccine is that the mechanisms of immune evasion and immune modulation by schistosomes are not clear. Unlike in some other infectious diseases, natural infection by schistosomes hardly induces

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Pseudomonas aeruginosa Triggers Macrophage Autophagy To Escape Intracellular Killing by Activation of the NLRP3 Inflammasome

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Assembly of the inflammasome has recently been identified to be a critical event in the initiation of inflammation. However, its role in bacterial killing remains unclear. Our study demonstrates that *Pseudomonas aeruginosa* infection induces the assembly of the NLRP3 inflammasome and the sequential secretion of caspase1 and interleukin-1β (IL-1β) in human macrophages. More importantly, activation of the NLRP3 inflammasome reduces the killing of *P. aeruginosa* in human macrophages, without affecting the generation of antimicrobial peptides, reactive oxygen species, and nitric oxide. In addition, our results demonstrate that *P. aeruginosa* infection increases the amount of the LC3-II protein and triggers the formation of autophagosomes in human macrophages. The *P. aeruginosa*-induced autophagy was enhanced by overexpression of NLRP3, ASC, or caspase1 but was reduced by knockdown of these core molecules of the NLRP3 inflammasome. Treatment with IL-1β enhanced autophagy in human macrophages. More importantly, IL-1β decreased the macrophage-mediated killing of *P. aeruginosa*, whereas knockdown of ATG7 or Beclin1 restored the IL-1β-mediated suppression of bacterial killing. Collectively, our study explores a novel mechanism employed by *P. aeruginosa* to escape from phagocyte killing and may provide a better understanding of the interaction between *P. aeruginosa* and host immune cells, including macrophages.

Pseudomonas aeruginosa is a Gram-negative bacterium which commonly exists in the environment and leads to diverse opportunistic infections. *P. aeruginosa* frequently infects immunocompromised individuals with tuberculosis and cancer and in particular infects those with cystic fibrosis (1). In recent years, the increased emergence of drug-resistant *P. aeruginosa* strains has brought a big challenge to traditional antibiotic therapies (2); therefore, efforts to understand the immune defense of the host against *P. aeruginosa* infection have attracted more attention.

Innate immune cells, such as macrophages and neutrophils, as well as the cytokines/chemokines secreted by these inflammatory cells, compose the first line of host defense. Most invading pathogens are engulfed by infiltrating phagocytes and then undergo intracellular elimination via either an oxygen-dependent or -independent bactericidal system, represented by reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively (3, 4), and via antimicrobial peptides, such as beta-defensins (BDs) and bactericidal/permeability-increasing protein (Bpi) (5). Recently, accumulating evidence demonstrates that autophagy is a protective strategy of host defense against a variety of intracellular pathogens, including *Shigella flexneri* (6), *Salmonella enterica* serovar Typhimurium (7), *Listeria monocytogenes* (8), and *Mycobacterium tuberculosis* (9, 10). However, the role of autophagy in the fight against extracellular pathogens like *P. aeruginosa* remains unclear.

Autophagy is an evolutionarily conserved catabolic mechanism which maintains cytoplasmic homeostasis by inducing the degradation of damaged organelles or misfolded proteins (11, 12). Initiation of autophagy is usually characterized by the formation of microtubule-associated protein light chain 3 (LC3) puncta, as well as the conversion of LC3-I into its lipidated form, LC3-II (13). Autophagy can be activated by starvation, rapamycin, as well as Toll-like receptor ligands and inflammatory cytokines like gamma interferon (IFN-γ) and tumor necrosis factor (TNF) (13). It is reported that *P. aeruginosa* can induce an autophagic response in macrophages (14) and mast cells (15). Nonetheless, the underlying mechanism still needs further investigation.

In addition to the induction of inflammatory cytokines and autophagy, bacterial infection also leads to the assembly of an intracellular complex called the inflammasome (16, 17). The inflammasome is one of the most important components in the innate immune and inflammatory response. The inflammasome is a large complex which is composed of multiple molecules, including adapter molecules like ASC and certain types of NOD-like receptors (NLRs) belonging to either the NLR family (e.g., NLRP1, NLRP3, or IPAF) or the PYHIN family (e.g., AIM2) (16). Enhanced expression of some NLRs like NLRP3 has been reported to be a critical checkpoint for inflammasome activation (17). Activation of the inflammasome finally leads to the cleavage of an inactive zymogen, procaspase1 (18). The active caspase1 then promotes the cleavage and secretion of interleukin-1β (IL-1β) and interleukin-18 (IL-18). The secreted IL-1β and IL-18 not only function as proinflammatory mediators in the immune defense against microbial infection but also have the potential to modulate

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Pseudomonas aeruginosa promotes autophagy to suppress macrophage-mediated bacterial eradication

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ABSTRACT

Objectives: To explore the role of autophagy on macrophage-mediated phagocytosis and intracellular killing of *Pseudomonas aeruginosa* (PA), a common extracellular bacterium which often causes various opportunistic infections.
Methods: Macrophages were infected with PA or stimulated with zymosan bioparticles. Autophagy was tested by fluorescent microscopy and Western blot for LC3. Phagocytosis and killing efficiency were assessed by plate count assay, flow cytometry or immunofluorescent staining. Phagocytic receptor expression, ROS generation and NO production were examined by PCR, flow cytometry and Griess reaction, respectively.
Results: PA infection induced autophagy activation in both mouse and human macrophages. Induction of autophagy by rapamycin or starvation significantly inhibited PA internalization by downregulating phagocytosis receptor expression, and suppressed intracellular killing of PA via reducing ROS and NO production in macrophages. While knockdown of autophagy molecules ATG7 or Beclin1 enhanced macrophage-mediated phagocytosis and intracellular killing of PA. Additionally, confocal microscopy data showed that induction of autophagy reduced the number of phagosomes and phagolysosomes in macrophages after stimulation with zymosan bioparticles.
Conclusions: Our study suggested that PA promotes autophagy to suppress macrophage-mediated bacterial phagocytosis and intracellular killing. These insights demonstrated a novel immune evasion mechanism employed by PA, which may provide potential therapeutic strategies of PA infectious diseases.

1. Introduction

Pseudomonas aeruginosa (PA) is a Gram-negative extracellular bacterium, which commonly exists in the environment and causes various opportunistic infectious diseases [1], such as keratitis in contact lens users [2], and nosocomial infections in patients with burning wounds, cystic fibrosis [3], or immunodeficiency [1]. Pathogenesis of these diseases largely results from bacterial virulence factors, such as exoenzyme ExoU, endotoxin lipopolysacharide (LPS) and exotoxin, which cause host cell death and tissue damage [4,5]. In recent decades, host antimicrobial immunity attracts more and more attention, because of the big challenge of drug-resistance in traditional antibiotic therapies [6].

Mononeuclear phagocyte system (MPS) is one of the most important components in the host immune system [7]. MPS system consists of the phagocytic cells located in reticular connective tissue, including monocytes, macrophages, as well as specialized macrophages like Kupffer cells, Langerhans cells, microglia and osteoclasts [7]. These cells recognize invading pathogens via distinct pattern recognition receptors expressed on the cell surface [8–10], and initiate the innate immune defense response. And engulf invading pathogens with the help of several phagocytic receptors on the cell surface [11], such as scavenger receptor (SR) [12], mannose receptor (MR) [13], Fc receptors for IgG (FcγR) [14], complement receptor (CR) [15], etc. After that, activated phagocytes produce a large amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [16,17], to kill the engulfed bacteria.

In addition to phagocytosis and intracellular killing, autophagy is another evolutionarily conserved cellular process in the innate immunity [18]. Autophagy promotes degradation of cytosolic components such as damaged organelles, misfolded proteins, and intracellular microorganisms via a lysosome-dependent pathway [19,20]. Initiation of autophagy is usually characterized by the formation of microtubule-

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Original Article

Pseudolaric acid B induces mitotic arrest and apoptosis in both 5-fluorouracil-sensitive and -resistant colorectal cancer cells

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ABSTRACT

5-fluorouracil (5-FU)-based chemotherapy is the main chemotherapeutic approach for colorectal cancer (CRC) treatment. Because chemoresistance occurs frequently and significantly limits CRC therapies, a novel agent is needed. Pseudolaric acid B (PAB), a small molecule derived from the Chinese medicinal herb "Tujinpi", exhibits strong cytotoxic effects on a variety of cancers. However, the detailed mechanisms by which PAB inhibits CRC cell growth and its potential role in overcoming 5-FU resistance have not been well studied. In this study, we showed that PAB significantly inhibited the viability of various CRC cell lines but induced minor cytotoxicity in normal cells. Both the *in vitro* and *in vivo* results showed that PAB induced proliferation inhibition, mitotic arrest and subsequently caspase-dependent apoptosis in both 5-FU-sensitive and -resistant CRC cells. Moreover, PAB was shown to interfere with CRC cell mitotic spindle apparatus and activate the spindle assembly checkpoint. Finally, CDK1 activity was involved in PAB-induced mitotic arrest and apoptosis in CRC cells. Taken together, these data reveal that PAB induces CRC cell mitotic arrest followed by apoptosis and overcomes 5-FU resistance *in vitro* and *in vivo*, suggesting that PAB may be a potential agent for CRC treatment, particularly for 5-FU-resistant CRC.

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide [1]. Surgery and chemotherapy are two major treatment options for CRC patients, and 5-fluorouracil (5-FU)-based chemotherapy is still the main chemotherapeutic approach in these patients. 5-FU-based chemotherapy has yielded a significant prolongation of survival. However, 5-FU resistance occurs frequently and results in a high level of treatment failure in CRC patients [2–4]. Therefore, novel agents are needed in order to increase the treatment efficacy and overcome 5-FU resistance.

Nearly 51% of the approved drugs in cancer therapeutics introduced from 1981 to 2014 were of natural origin [5]. And an increasing number of traditional Chinese medicines from natural products have been recently verified to have strong anti-cancer effects and induce fewer side effects [5,6]. Therefore, the exploration of efficient anti-cancer drugs from Chinese medicines has become an attractive strategy. The Chinese medicinal herb "Tujinpi" is from the root and trunk bark of a tree named *Pseudolarix kaempferi* Gorden (Pinaceae) from central China [7]. This medicinal herb has been used to treat skin inflammation for several centuries in China. The diterpene acid pseudolaric acid B (PAB), shown in Fig. S1, is a major biologically active component in

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for 2014. This prevalence is increasing compared to that described in France in 2012 (14%). We found 35 A→G substitutions at position 2058 or 2059, two A2062T mutations and one A2059C mutation (Table) (*1*,*9*). Notably, in patients 15 and 33, who were infected with strains with macrolide resistance–associated mutations, *M. genitalium* infection was unsuccessfully treated with azithromycin, with treatment failures after azithromycin (1 g) and extended azithromycin (1.5 g for 5 d), but moxifloxacin treatment was effective. Patient 15 had been treated 1 year earlier with azithromycin (1 g) for nongonococcal urethritis.

Among the 168 patients whose isolates were examined for the 23S rRNA, *gyr*A, and *par*C genes, strains from 2 patients (patients 3 and 6) had both macrolide- and fluoroquinolone-associated mutations (1.2%; 95% CI 0.33%–4.24%). Both patients received azithromycin (1 g), and patient 6 received additional azithromycin (1.5 g) after failure of azithromycin (1 g). Patient 6 experienced azithromycin failure again after the extended regimen. *M. genitalium* multidrug resistance is described in France at a prevalence of 1.2%, lower than prevalence described in Australia (7.5%) (*7*) and Japan (30.8%) (*10*).

In conclusion, *M. genitalium* fluoroquinolone resistance is emerging in France, with a prevalence of 6% in 2013–2014. Further, macrolide resistance also increased during this period, to a rate of 17.2%. Patients infected with *M. genitalium* strains containing both macrolide and fluoroquinolone resistance mutations associated with therapeutic failure raise concerns about untreatable *M. genitalium* infections.

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References

1. Touati A, Peuchant O, Jensen JS, Bébéar C, Pereyre S. Direct detection of macrolide resistance in *Mycoplasma genitalium* isolates from clinical specimens from France by use of real-time PCR and melting curve analysis. J Clin Microbiol. 2014;52:1549–55. http://dx.doi.org/10.1128/JCM.03318-13
2. Couldwell DL, Tagg KA, Jeoffreys NJ, Gilbert GL. Failure of moxifloxacin treatment in *Mycoplasma genitalium* infections due to macrolide and fluoroquinolone resistance. Int J STD AIDS. 2013;24:822–8. http://dx.doi.org/10.1177/0956462413502008
3. Pond MJ, Nori AV, Witney AA, Lopeman RC, Butcher PD, Sadiq ST. High prevalence of antibiotic-resistant *Mycoplasma genitalium* in nongonococcal urethritis: the need for routine testing and the inadequacy of current treatment options. Clin Infect Dis. 2014;58:631–7. http://dx.doi.org/10.1093/cid/cit752
4. Shimada Y, Deguchi T, Nakane K, Masue T, Yasuda M, Yokoi S, et al. Emergence of clinical strains of *Mycoplasma genitalium* harbouring alterations in ParC associated with fluoroquinolone resistance. Int J Antimicrob Agents. 2010;36:255–8. http://dx.doi.org/10.1016/j.ijantimicag.2010.05.011
5. Deguchi T, Yasuda M, Horie K, Seike K, Kikuchi M, Mizutani K, et al. Drug resistance-associated mutations in *Mycoplasma genitalium* in female sex workers, Japan. Emerg Infect Dis. 2015;21:1062–4. http://dx.doi.org/10.3201/eid2106.142013
6. Bissessor M, Tabrizi SN, Twin J, Abdo H, Fairley CK, Chen MY, et al. Macrolide resistance and azithromycin failure in a *Mycoplasma genitalium*–infected cohort and response of azithromycin failures to alternative antibiotic regimens. Clin Infect Dis. 2015;60:1228–36. http://dx.doi.org/10.1093/cid/ciu1162
7. Tagg KA, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and macrolide resistance-associated mutations in *Mycoplasma genitalium.* J Clin Microbiol. 2013;51:2245–9. http://dx.doi.org/10.1128/JCM.00495-13
8. Kikuchi M, Ito S, Yasuda M, Tsuchiya T, Hatazaki K, Takanashi M, et al. Remarkable increase in fluoroquinolone-resistant *Mycoplasma genitalium* in Japan. J Antimicrob Chemother. 2014;69:2376–82. http://dx.doi.org/10.1093/jac/dku164
9. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in *Mycoplasma genitalium*-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis. 2008;47:1546–53. http://dx.doi.org/10.1086/593188
10. Deguchi T, Kikuchi M, Yasuda M, Ito S. Multidrug-resistant *Mycoplasma genitalium* is increasing. Clin Infect Dis. 2016;62:405–6. http://dx.doi.org/10.1093/cid/civ898

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Possible Transmission of *mcr-1*–Harboring *Escherichia coli* between Companion Animals and Human

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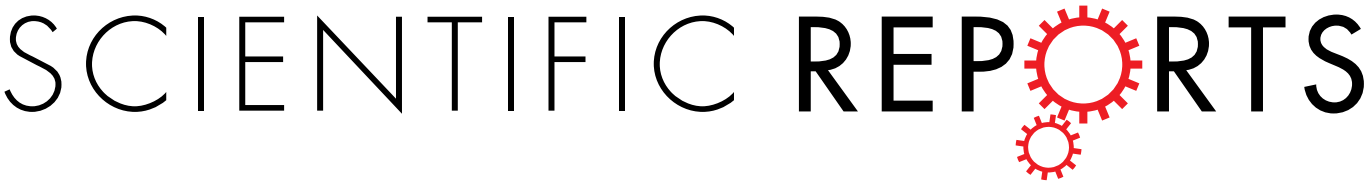
To the Editor: Plasmid-mediated, colistin-resistance mechanism gene *mcr-1* was first identified in *Escherichia coli* isolates from food, food animals, and human patients in November 2015 (*1*). Reports on detection of *mcr-1* in *Enterobacteriaceae* from humans and food animals



OPEN

Phenotypic and functional characteristics of IL-21-expressing CD8$^+$ T cells in human nasal polyps

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Although CD4$^+$ T cells are recognized to play an important role in the inflammatory response of nasal polyps (NPs), the biological functions of CD8$^+$ T cells in polypogenesis remain unclear. In this study, we analyzed cell markers, cytokine expression and transcription factors in IL-21-expressing CD8$^+$ T cells in polyp tissues of NP patients. The results showed that the majority of IL-21-producing CD8$^+$ T cells were effector memory cells and they co-expressed IFN-γ. IL-21-expressing CD8$^+$ T cells in polyp tissues expressed higher CXCR5, PD-1, and ICOS levels than cells in control tissues and showed significantly higher T-bet and Bcl-6 expression levels compared with IL-21$^-$CD8$^+$ T cells. Purified polyp CD8$^+$ T cells promoted IgG production from isolated polyp B cells *in vitro*, and recombinant IL-12 modulated the expression of IL-21, IFN-γ and CD40L in purified polyp CD8$^+$ T cells. Moreover, the percentage of IL-21$^+$CD8$^+$ T cells in polyp tissues was positively correlated with endoscopic and CT scan scores in NP patients. These findings indicated that polyp CD8$^+$ T cells, by co-expressing IL-21 and IFN-γ and other markers, display a Tfh cell functionality, which is associated with the clinical severity of NP patients.

Nasal polyps (NPs) is a chronic inflammatory disease of the upper airway and commonly arise from the paranasal sinuses[1]. It remains one of the most challenging diseases in the field of rhinology due to its complex etiology and frequent recurrence. NPs is thought as a multifactorial disease with several different etiological factors, including infection and allergy[2]. T lymphocytes are critical for the development of sinonasal inflammation, both CD4$^+$ T cells (including Th1, Th2, Th17 and Treg cells) and their cytokines have been reported to display different immune effects in the local inflammatory reaction of NPs[3–6]. Moreover, regional (i.e., Asian vs. Western countries) and/or racial differences in the roles of various Th cell subsets in the local inflammatory reaction in NP tissues have been reported[7]. Similar to the subsets of CD4$^+$ T cells, the subsets of CD8$^+$ T cells, including Tc1, Tc2, and Tc17 cells, and their interrelated cytokines have been shown to play an important role in the adaptive immune response to pathogens in various infectious and inflammatory diseases, including HIV infection[8], rheumatic diseases[9] and tuberculosis[10]. In airway diseases, CD8$^+$ T cells were found to suppress allergen-induced late airway responses, inhibit airway eosinophilia through secretion of IFN-γ and mediate pulmonary alveolitis and inflammation[11,12]. However, the clinical significance of CD8$^+$ T cells in NPs remains to be elucidated. Interleukin-21 (IL-21), a member of the common-γ chain (γc) family of cytokines, has the ability to act on multiple cells of the immune system[13]. Consistent with its broad effects, it has become clear that not only does IL-21 regulate normal lymphoid development and function, but it also serves critical roles in inflammatory, allergic, autoimmune and neoplastic diseases[14]. However, it is unknown whether IL-21 is involved in the sinonasal inflammatory responses of NP patients.

Our previous studies showed that polyp tissues contained significantly higher IL-21 production compared with control mucosa tissues and that IL-21 level positively correlated with polyp size and surgical recurrence of NP patients[15]. IL-21 is normally thought to be produced by various subsets of CD4$^+$ T cells and NKT cells[16]. In the present study, we found that CD8$^+$ T cells from polyp tissues produced high levels of IL-21 as well. Some of

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REVIEW ARTICLE

One Health in China

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As a result of rapid economic growth over the previous three decades, China has become the second largest economy worldwide since 2010. However, as a developing country with the largest population, this rapid economic growth primarily based on excessive consumption and waste of resources. Thus, China has been facing particularly severe ecological and environmental problems in speeding up industrialization and urbanization. The impact of the health risk factors is complex and difficult to accurately predict. Therefore, it is critical to investigate potential threats in the context of the human-animal-environment interface to protect human and animal health. The "One Health" concept recognizes that human health is connected to animal and environmental health. This review primarily discusses specific health problems in China, particularly zoonoses, and explains the origin and development of the One Health approach, as well as the importance of a holistic approach in China.

Keywords: *One Health approach*; *human–animal–environment interface*; *zoonosis*; *food safety*; *antimicrobial resistance*

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Consistent with the basic national policy of economic development as the central task, in combination with population growth,urbanization and industrial expansion, high energy consumption and serious pollution have made the ecological system more fragile in China. In the previous three decades, the contradictions among the environment, resources and economic growth have become increasingly prominent. The natural ecology has deteriorated,which not only restricts the long-term economic development but also impacts human interests and even threatens the survival of mankind. The balance between economic development and ecological conservation in China has become a critical issue in recent decades (1). Visible effects, such as emerging infectious diseases, SARS and MERS, indicate the necessity for close collaborations which is the advocacy by One Health include the national and local government, non-government agency, academia, research institute, interdisciplinary science community, the private sector, civil society and other stakeholder. One Health is a interdisciplinary approach suited to address health issues at the human-animal-environment interface (2). One Health and Ecohealth (Ecosystem health), another holistic approach, despite the different origins, they appear to have increasing alignment regarding the aspects of vision and goals in recent years. However, compared with Ecohealth, One Health focuses more on network building, that's why we only focus on One Health here.

Health problems in China

Increasing health problems at the human–animal–environment interface in China

Currently, China has the largest population in the world, which accounts for 19.7% of the world's total population (1.393 billion/7.126 billion) (3). The population growth rate has declined since the 1970s, when the national 'One-Child Policy' was implemented. Nevertheless, the population in 1997 doubled compared with 1949 because of the momentum of population growth. According to the 2013 International Statistics Yearbook (4), China has also experienced substantial economic growth over the previous three decades. However, increasing demands or depletions of natural resources are always in companion

†These authors contributed equally to this work.

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Infectious Diseases of Poverty

SCOPING REVIEW Open Access

Nucleic acid detection in the diagnosis and prevention of schistosomiasis

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Abstract

Schistosomiasis is an important zoonotic parasitic disease that causes serious harms to humans and animals. Surveillance and diagnosis play key roles in schistosomiasis control, however, current techniques for surveillance and diagnosis of the disease have limitations. As genome data for parasites are increasing, novel techniques for detection incorporating nucleotide sequences are receiving widespread attention. These sensitive, specific, and rapid detection methods are particularly important in the diagnosis of low-grade and early infections, and may prove to have clinical significance. This paper reviews the progress of nucleic acid detection in the diagnosis and prevention of schistosomiasis, including such aspects as the selection of target genes, and development and application of nucleic acid detection methods.

Keywords: *Schistosoma japonicum*, *S. mansoni*, *S. haematobium*, Diagnosis, Nucleic acid detection

Multilingual abstracts

Please see Additional file 1 for translations of the abstract into the six official working languages of the United Nations.

Introduction

Schistosomiasis or bilharzia, a serious parasitic disease (third in importance to malaria and amoebiasis) caused by blood flukes, continues to plague many developing countries in the tropics. According to the World Health Organization (WHO) [1], in 2012 there were still 240 million patients with schistosomiasis globally, of which 45.8 % were children aged between 5 and 14 years, with 93 % of patients living in Africa. These statistics show little change from those reported previously [2, 3], indicating that schistosomiasis has not yet been effectively controlled.

Human schistosomiasis is mainly caused by three species of schistosomes: *Schistosoma japonicum, S. mansoni*, and *S. haematobium*. A schistosome has a complex life cycle. The adult worms parasitize in the host's blood system, and their eggs are excreted through human feces or urine. Then, the eggs hatch into miracidia in freshwater sources and quickly penetrate into intermediate host snails, which can subsequently become sporocysts and develop into cercaria by asexual reproduction. Humans acquire the infection after direct contact with cercaria released from infected snails. Different species of schistosomes share similar epidemic patterns.

Accurate surveillance and diagnosis play key roles in the prevention and control of schistosomiasis. Firstly, information on snail infection and cercariae distribution is required in order to evaluate the risk of infection. Both cercarial shedding and microscopic examination of snails are conventional diagnosis methods. However, the positive rate of snails achieved using etiological methods significantly decreases in endemic areas [4]. Moreover, an etiological method is unable to distinguish between the different species of cercaria. The Kato-Katz technique is still the gold standard in schistosomiasis diagnosis. This technique is cheap, convenient, and qualitative [5], but is limited in the diagnosis of low-grade and prepatent infections, as well as in evaluating drug therapeutic effects. The vast increase in genome data for parasites offer valuable insights into the development of novel drug candidates and more accurate diagnostic tools [6]. Some researchers have reported that certain nucleic acid sequences derived from schistosomes can be detected in human sera [7, 8], suggesting that nucleic acid detection

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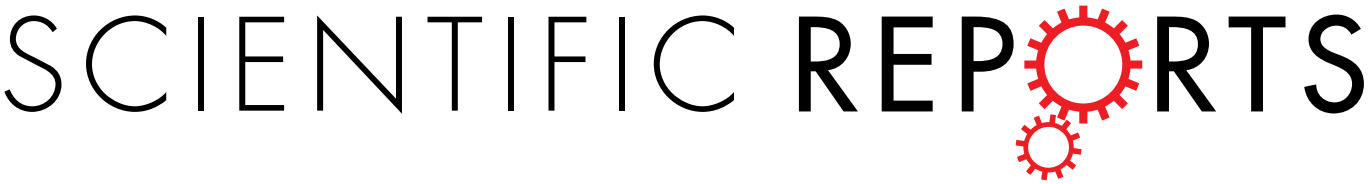

OPEN

Nuclear Factor Erythroid 2-related Factor 2 Deficiency Exacerbates Lupus Nephritis in B6/*lpr* mice by Regulating Th17 Cell Function

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Lupus nephritis (LN) is the major clinical manifestation of systemic lupus erythematosus. LN is promoted by T helper 17 (Th17) cells, which are the major pro-inflammatory T cell subset contributing to autoimmunity regulation. Nuclear factor erythroid 2-related factor 2 (NRF2) is critical for suppressing reactive oxygen species (ROS) and relieving oxidant stress by regulating antioxidant gene expression. Previous studies have demonstrated that *Nrf2* deficiency promotes drug-induced or spontaneous LN. However, whether NRF2 regulates Th17 function during LN development is still unclear. In this study, we introduced *Nrf2* deficiency into a well-known LN model, the B6/*lpr* mouse strain, and found that it promoted early-stage LN with altered Th17 activation. Th17 cells and their relevant cytokines were dramatically increased in these double-mutant mice. We also demonstrated that naïve T cells from the double-mutant mice showed significantly increased differentiation into Th17 cells *in vitro*, with decreased expression of the Th17 differentiation suppressor *Socs3* and increased phosphorylation of STAT3. Our results demonstrated that *Nrf2* deficiency promoted Th17 differentiation and function during LN development. Moreover, our results suggested that the regulation of Th17 differentiation via NRF2 could be a therapeutic target for the treatment of subclinical LN patients.

Lupus nephritis (LN) is the major clinical manifestation of systemic lupus erythematosus (SLE). SLE is a complicated autoimmune disease that is characterised by the production of autoantibodies, systemic inflammation, and damage to vessels and organs[1]. SLE is a multifactorial disease caused by genetic and environmental factors. Many SLE susceptibility genes are responsible for maintaining immune tolerance and homeostasis, such as antigen processing and presentation, clearance of apoptotic debris, leukocyte cell surface receptors, and cell signalling and gene transcription molecules[2–4]. The pathogenesis of LN involves abnormal B and T cell responses, which promote the production of autoantibodies and immune complex deposits in the kidney and other organs[5]. Recent studies have found that T cells are primarily responsible for the pathogenesis of LN, including the regulation of B cell responses and the production of autoantibodies, the modulation and differentiation of T helper (Th) cell and effector cell expansion and function, and the activation of macrophages and natural killer cell functions[5,6].

Th cells, which are central regulators of adaptive immune responses, play a crucial role in the pathogenesis of SLE by regulating the interactions between other cells and contributing to the production of immunomodulatory cytokines[4]. Upon antigen stimulation, naïve $CD4^+$ T cells differentiate into different lineages of Th cells, including Th1, Th2, Th17, Th9, Th22, and Treg cells, according to the types of cytokines they are stimulated by[7]. In particular, Th17 cells, which produce the pro-inflammatory cytokine interleukin (IL)-17, are important for the pathogenesis of SLE[8–10]. Patients with SLE have higher levels of IL-17 in serum compared to healthy controls, and an increased amount of IL-17-producing T cells in peripheral blood[11–13]. B6/*lpr* mice deficient in IL-23 signalling were resistant to the development of LN and showed deficient Th17 development[14]. Th17 also co-regulates the

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Niclosamide inhibits lytic replication of Epstein-Barr virus by disrupting mTOR activation

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ABSTRACT

Infection with the oncogenic γ-herpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) cause several severe malignancies in humans. Inhibition of the lytic replication of EBV and KSHV eliminates the reservoir of persistent infection and transmission, consequently preventing the occurrence of diseases from the sources of infection. Antiviral drugs are limited in controlling these viral infectious diseases. Here, we demonstrate that niclosamide, an old anthelmintic drug, inhibits mTOR activation during EBV lytic replication. Consequently, niclosamide effectively suppresses EBV lytic gene expression, viral DNA lytic replication and virion production in EBV-infected lymphoma cells and epithelial cells. Niclosamide exhibits cytotoxicity toward lymphoma cells and induces irreversible cell cycle arrest in lytically EBV-infected cells. The ectopic overexpression of mTOR reverses the inhibition of niclosamide in EBV lytic replication. Similarly, niclosamide inhibits KSHV lytic replication. Thus, we conclude that niclosamide is a promising candidate for chemotherapy against the acute occurrence and transmission of infectious diseases of oncogenic γ-herpesviruses.

1. Introduction

Two natural human oncogenic γ-herpesviruses, Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), cause several types of severe malignancies (Ganem, 2010; Kutok and Wang, 2006). These viruses have two alternative lifecycles after their DNA genomes enter into the cellular nucleus: default latency and a small portion of lytic replication. Lytic replication provides a reservoir of infectious virion particles for expansion and transmission (Ganem, 2010; Kenney and Mertz, 2014). Thus, the blockade of lytic replication could effectively prevent the incidence of infection and diseases from their sources of infection. Although there are many antiviral drugs available (Siakallis et al., 2009; Skorenski and Sienczyk, 2014), few have been assessed in treating acute infection and lytic replication of these viruses.

Homologous EBV and KSHV share a high similarity of their viral DNA genomes and viral gene products (Damania, 2004; Nicholas, 2000). Consequently, these viruses employ a variety of common cellular pathways to facilitate their infection, replication and maintenance of viral genomes as well as tumorigenesis (Collins and Medveczky, 2002; Damania and Jung, 2001; de Oliveira et al., 2010; Filippakis et al., 2010; Hayward et al., 2006; Noguchi et al., 2007; Stevenson, 2004). Disruption of these pathways by inhibitors mostly leads to the inhibition of their infection and further pathogenesis; however, therapeutic clinical applications remain unavailable.

Niclosamide is one of the World Health Organization's essential medicines and is classified as an effective anthelmintic drug to treat worm infections, especially tapeworm infections (Craig and Ito, 2007). Niclosamide is also effective against intractable drug-resistant bacterial infections (Costabile et al., 2015; de Carvalho et al., 2011; Imperi et al., 2013; Rajamuthiah et al., 2015). As niclosamide inhibits mTORC1 signaling through disruption of cellular pH homeostasis (Balgi et al., 2009; Fonseca et al., 2012) and lysosome inhibition-induced Rag-mTORC1 signaling (Li et al., 2013), it can be used as a preclinical inducer of autophagy. Moreover, niclosamide uncouples mitochondrial respiration and disrupts cellular metabolism, which provides a potential approach for treating type 2 diabetes (Tao et al., 2014).

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Article

Myeloid-derived suppressor cells are essential for maintaining feto-maternal immunotolerance via STAT3 signaling in mice

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ABSTRACT

Maternal immune system tolerance to the semiallogeneic fetus is essential for a successful pregnancy; however, the mechanisms underlying this immunotolerance have not been fully elucidated. Here, we demonstrate that myeloid-derived suppressor cells play an important role in maintaining feto-maternal tolerance. A significant expansion of granulocytic myeloid-derived suppressor cells was observed in multiple immune organs and decidual tissues from pregnant mice. Pregnancy-derived granulocytic myeloid-derived suppressor cells suppressed T cell responses in a reactive oxygen species-dependent manner and required direct cell–cell contact. Mechanistic studies showed that progesterone facilitated differentiation and activation of granulocytic myeloid-derived suppressor cells, mediated through STAT3 signaling. The STAT3 inhibitor JSI-124 and a specific short hairpin RNA completely abrogated the effects of progesterone on granulocytic myeloid-derived suppressor cells. More importantly, granulocytic myeloid-derived suppressor cell depletion dramatically enhanced the abortion rate in normal pregnant mice, whereas adoptive transfer of granulocytic myeloid-derived suppressor cells clearly reduced the abortion rate in the CBA/J X DBA/2J mouse model of spontaneous abortion. These observations collectively demonstrate that granulocytic myeloid-derived suppressor cells play an essential role in the maintenance of fetal immunotolerance in mice. Furthermore, our study supports the notion that in addition to their well-recognized roles under pathologic conditions, myeloid-derived suppressor cells perform important functions under certain physiologic circumstances. *J. Leukoc. Biol.* **100: 499–511; 2016.**

Abbreviations: BM = bone marrow, CM-H_2DCFDA = chloromethyl derivative of 2′,7′-dichlorodihydrofluorescein diacetate, DC = dendritic cell, dNK = decidual NK, E = embryonic day, E2 = 17-β estridol, ER = estrogen receptor, F = forward, G-MDSC = granulocytic myeloid-derived suppressor cell, HBS = HEPES-buffered saline, L-NMMA = N-α-methyl-(L)-arginine, M-MDSC = monocytic myeloid-derived suppressor cell,

(*continued on next page*)

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

Introduction

Pregnancy constitutes a major challenge to the maternal immune system, and tolerance to fetal alloantigens must be maintained to avoid allograft rejection [1]. The possibility that the fetus is shielded from the maternal immune system by a physical barrier has been excluded, because studies have shown that placental cells (fetal) penetrate deep into the uterine mucosa (maternal) and that cell–cell contact is established between the fetus and the maternal immune system [2]. Studies from both mouse and human pregnancies have demonstrated that the maternal immune system actively responds to fetal antigens [3–5].

Several distinct mechanisms may explain feto-maternal tolerance. The recruitment and differentiation of a variety of immune cells in the decidua play important roles in maintaining maternal tolerance against fetal antigens [6, 7]. For example, $CD56^+CD16^-$ dNK cells accumulate at the feto-maternal interface during human pregnancy through local generation and CXCR-mediated recruitment of a NK cell lineage [8]. The components of the uterine niche may trigger decidua-specific functional plasticity in the phenotype of dNK cells to promote fetal tolerance, although additional mechanisms may exist [9]. Clonal deletion or suppression of fetal antigen-reactive T cells also contributes to fetal tolerance, possibly through an interaction between inhibitory costimulatory receptors on effector T cells and the corresponding ligands on trophoblasts and decidual stromal cells [1, 10]. Additionally, T_{regs} are active players in the maintenance of fetal tolerance [11]. Studies have demonstrated that the frequency of T_{regs} is elevated in both mouse and human

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RESEARCH ARTICLE

Mycobacterium tuberculosis-Specific IL-21$^+$IFN-γ$^+$CD4$^+$ T Cells Are Regulated by IL-12

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Abstract

In the current study of *Mycobacterium tuberculosis* (MTB)-specific T and B cells, we found that MTB-specific peptides from early secreted antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) induced the expression of IL-21 predominantly in CD4$^+$ T cells. A fraction of IL-21-expressing CD4$^+$ T cells simultaneously expressed Th1 cytokines but did not secrete Th2 or Th17 cytokines, suggesting that MTB-specific IL-21-expressing CD4$^+$ T cells were different from Th1, Th2 and Th17 subpopulations. The majority of MTB-specific IL-21-expressing CD4$^+$ T cells co-expressed IFN-γ and IL-21$^+$IFN-γ$^+$CD4$^+$ T cells exhibited obviously polyfunctionality. In addition, MTB-specific IL-21-expressing CD4$^+$ T cells displayed a CD45RO$^+$CD62L^{low}CCR7^{low}CD40L^{high}ICOS^{high} phenotype. Bcl-6-expression was significantly higher in IL-21-expressing CD4$^+$ T cells than IL-21$^-$CD4$^+$ T cells. Moreover, IL-12 could up-regulate MTB-specific IL-21 expression, especially the frequency of IL-21$^+$IFN-γ$^+$CD4$^+$ T cells. Taken together, our results demonstrated that MTB-specific IL-21$^+$IFN-γ^{+-} CD4$^+$ T cells from local sites of tuberculosis (TB) infection could be enhanced by IL-12, which have the features of both Tfh and Th1 cells and may have an important role in local immune responses against TB infection.

Introduction

Tuberculosis (TB) is one of the most ancient diseases of mankind and currently remains a leading cause of death from infectious disease worldwide [1–3]. The incidence of TB has increased over the past few years for reasons such as inadequate preventative efforts, incorrect or inappropriate medication, the emergence of drug-resistant strains of *Mycobacterium tuberculosis* (MTB) and the prevalence of human immunodeficiency virus (HIV) infection [4–6].

Cell-mediated immunity is known to be crucial for protection against TB and most studies have shown that CD4$^+$ and CD8$^+$ T cells are essential for protective immunity [7–10]. We have been committed to studying MTB-specific effector and memory CD4$^+$ T cells, including Th1,

miR-214/199a/199a* cluster levels predict poor survival in hepatocellular carcinoma through interference with cell-cycle regulators

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ABSTRACT

Aims: To identify the clinical and functional association of miR-214/199a/199a* cluster in human hepatocellular carcinoma (HCC) and to clarify the mechanism of miR-214.

Methods: Kaplan-Meier and Cox proportional regression analyses were used to determine the association of miR-214/199a/199a* cluster levels with the survival of HCC patients. The role of miR-214 in regulating HCC cell proliferation was studied with miR-214 mimics/inhibitor-treated cells. Furthermore, the inhibition effect of miR-214 on E2F2, cyclin-dependent kinase (CDK) 3 and CDK6 expression was assessed in HCC cell lines with miR-214 mimics/inhibitors to increase/decrease miR-214 expression. Direct binding of miR-214 to the 3′-untranslated regions of E2F2, CDK3, and CDK6 was verified by dual-luciferase reporter assay.

Results: In analyzing HCC clinical specimens and cell lines, we discovered a uniform decrease in miR-214/199a/199a* expression in comparison with noncancerous tissue or normal liver epithelial cell lines. Higher miR-214 levels were related with improved patient survival. Overexpression of miR-214 in HCC cells inhibited proliferation by inducing G1-S checkpoint arrest. Conversely, RNA interference–mediated silencing of miR-214 promoted cell-cycle progression and accelerated the proliferation of HCC cells. E2F2, CDK3 and CDK6 were each directly targeted for inhibition by miR-214, and restoring their expression reversed miR-214 inhibition of cell-cycle progression. The relationship between expression of miR-214 and its targets was confirmed in HCC tumor xenografts and clinical specimens.

Conclusions: Our results demonstrate that miR-214 has tumor-suppressive activity in HCC through inhibition of E2F2, CDK3 and CDK6.

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microRNA-146a promotes mycobacterial survival in macrophages through suppressing nitric oxide production

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Macrophages play a crucial role in host innate anti-mycobacterial defense, which is tightly regulated by multiple factors, including microRNAs. Our previous study showed that a panel of microRNAs was markedly up-regulated in macrophages upon mycobacterial infection. Here, we investigated the biological function of miR-146a during mycobacterial infection. miR-146a expression was induced both *in vitro* and *in vivo* after *Mycobacterium bovis* BCG infection. The inducible miR-146a could suppress the inducible nitric oxide (NO) synthase (iNOS) expression and NO generation, thus promoting mycobacterial survival in macrophages. Inhibition of endogenous miR-146a increased NO production and mycobacterial clearance. Moreover, miR-146a attenuated the activation of nuclear factor κB and mitogen-activated protein kinases signaling pathways during BCG infection, which in turn repressed iNOS expression. Mechanistically, miR-146a directly targeted tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) at post-transcriptional level. Silencing TRAF6 decreased iNOS expression and NO production in BCG-infected macrophages, while overexpression of TRAF6 reversed miR-146a-mediated inhibition of NO production and clearance of mycobacteria. Therefore, we demonstrated a novel role of miR-146a in the modulation of host defense against mycobacterial infection by repressing NO production via targeting TRAF6, which may provide a promising therapeutic target for tuberculosis.

Tuberculosis (TB) is still a leading public health threat worldwide with high morbidity and mortality[1]. In 2014, World Health Organization reported that an estimated 9.0 million incident cases of TB occurred, with 1.5 million deaths caused by the disease[2]. *Mycobacterium tuberculosis* (MTB) is the causative agent responsible for TB, and infects approximately one-third of the human population globally. However, only about 10% of infected individuals develop active TB, while the remaining 90% cases exhibit latent infection, indicating a critical role of the host immunity in the containment of MTB infection[3].

Macrophages act as the first line of host immune defense against MTB[4]. The infectious bacilli are inhaled as aerosol particles, and phagocytosed by resident macrophages. Invading MTB are recognized by macrophages through pattern-recognition receptors (PRRs), which trigger innate immune defense, and subsequently initiate adaptive immune responses to pathogenic MTB[5–7]. During TB infection, Toll-like receptors (TLRs) are involved in host innate recognition of MTB[8–11]. Engagement with cognate ligands activates the TLR signaling pathway via the adaptor myeloid differentiation primary response gene 88 (MyD88)[12]. Sequentially, tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) relays MyD88-dependent TLR signaling and leads to the activation of downstream nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs, including JNK, ERK and p38) pathways[13], which ultimately results in the production of inflammatory cytokines and direct antimicrobial mediators, such as TNF and nitric oxide (NO)[14].

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Research Paper

Mesenchymal marker expression is elevated in Müller cells exposed to high glucose and in animal models of diabetic retinopathy

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ABSTRACT

Müller cells are retinal glial cells and exhibit a fibroblast-like phenotype and ability to migrate in diabetic retinopathy (DR). However, expression of mesenchymal markers, which promote fibrosis in various organs, has not been characterized in the diabetic retina. We examined changes in the expression of these markers in Müller cells exposed to high glucose and in animal models of diabetic retinopathy. High glucose conditions increased mesenchymal maker expression and migration in Müller cells. Snail, N-cadherin, Vimentin, β-catenin, and α-smooth muscle actin (α-SMA) levels were all dramatically increased in retinas from humans with diabetic retinopathy (DR) and from DR mouse models. In addition, Snail overexpression increased the expression of connective tissue growth factor (CTGF) and fibronectin, while Snail knockdown attenuated high glucose-induced increases in fibronectin and CTGF expression. These results demonstrate for the first time that mesenchymal markers are upregulated in retinas from a diabetic mouse model, and that Snail and N-cadherin levels are also increased in Müller cells exposed to high glucose. This suggests mesenchymal proteins may play a crucial role in the development of DR.

INTRODUCTION

Diabetic retinopathy (DR) is a severe complication of diabetes and the leading cause of blindness among working adults worldwide [1]. DR is classified as either non-proliferative (non-PDR) or proliferative (PDR) [2]. The main pathogenic features of PDR are preretinal neovascularization and the formation of fibrovascular membranes at the vitreoretinal interface. The presence of fibrovascular tissue often results in severe visual impairment due to vitreous hemorrhages and/or tractional retinal detachment [3]. Although retinal neovascularization has been considered the main characteristic of PDR, the fibrogenic process that occurs after new vessels are formed

PLOS | ONE

RESEARCH ARTICLE

Memory-Like Antigen-Specific Human NK Cells from TB Pleural Fluids Produced IL-22 in Response to IL-15 or *Mycobacterium tuberculosis* Antigens

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Abstract

Our previous result indicated that memory-like human natural killer (NK) cells from TB pleural fluid cells (PFCs) produced large amounts of IFN-γ in response to *Bacille Calmette Guerin* (BCG). Furthermore, recent studies have shown that human lymphoid tissues harbored a unique NK cell subset that specialized in production of interleukin (IL)-22, a proinflammatory cytokine that mediates host defense against pathogens. Yet little information was available with regard to the properties of IL-22 production by memory-like human NK cells. In the present study, we found that cytokines IL-15 induced and IL-12 enhanced the levels of IL-22 by NK cells from TB PFCs. In addition, IL-22 but not IL-17 was produced by NK cells from PFCs in response to BCG and *M.tb*-related Ags. More importantly, the subset of specific IL-22-producing NK cells were distinct from IFN-γ-producing NK cells in PFCs. $CD45RO^+$ or $CD45RO^-$ NK cells were sorted, co-cultured with autologous monocytes and stimulated with BCG for the production of IL-22. The result demonstrated that $CD45RO^+$ but not $CD45RO^-$ NK cells produced significantly higher level of IL-22. Anti-IL-12Rβ1 mAbs (2B10) partially inhibit the expression of IL-22 by NK cells under the culture with BCG. Consistently, BCG specific IL-22-producing NK cells from PFCs expressed $CD45RO^-{}^{high}NKG2D^{high}$granzyme B^{high}. In conclusion, our data demonstrated that memory-like antigen-specific $CD45RO^+$ NK cells might participate in the recall immune response for *M. tb* infection via producing IL-22, which display a critical role to fight against *M. tb*.

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BMC Cancer

RESEARCH ARTICLE Open Access

MEK5 overexpression is associated with the occurrence and development of colorectal cancer

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Abstract

Background: Mitogen/extracellular signal-regulated kinase kinase-5 (MEK5) has been confirmed to play a pivotal role in tumor carcinogenesis and progression. However, few studies have investigated the role of MEK5 in colorectal cancer (CRC).

Methods: MEK5 expression was determined by immunohistochemistry (IHC) in tissue microarrays (TMAs) containing 2 groups of tissues, and western blotting was used to confirm MEK5 expression in 8 cases of primary CRC tissues and paired normal mucosa. RNA interference was used to verify the biological function of MEK5 gene in the development of CRC.

Results: IHC revealed the expression of MEK5 was higher in tumor tissues (38.1 %), compared with adjacent normal tissue (8.3 %). Western blot showed that, MEK5 expression was upregulated in CRC tumor tissues compared with normal tissue. Analysis of clinical pathology parameters indicated MEK5 overexpression was significantly correlated with the depth of invasion, lymph node metastasis, distant metastasis and histological grade. Survival analysis revealed that MEK5 overexpression negatively correlated with cancer-free survival (hazard ratio 1.64, $P = 0.017$). RNA interference-mediated knockdown of MEK5 in SW480 colon cancer cells decreased their proliferation, division, migration and invasiveness in vitro and slowed down tumors growth in mice engrafted with the cells.

Conclusion: MEK5 plays an important role in CRC progression and may be a potential molecular target for the treatment of CRC.

Keywords: MEK5, Colorectal cancer, Univariate analyses, RNA interference, Tumor growth

Background

Colorectal cancer (CRC) is a common malignant disease and remains one of the leading causes of cancer mortality worldwide [1]. With the development of China's economy, the incidence of CRC in China is increasing and now causes a substantial cancer burden in China, particularly in the more developed areas such as Guangdong and Shanghai [2–4]. The carcinogenesis of CRC is often a multistep process and possibly consequent of a complex interaction between multiple factors, both endogenous and environmental stressors [5]. The environmental stressors such as drinking and smoking could lead to activation of many critical molecular pathways, such as mitogen-activated protein kinases (MAPKs) [6], and the Wnt/Wingless signaling pathway [7], eliciting a variety of biological responses.

MAP kinase kinases (MEKs/MAPKKs) represent a family of protein kinases upstream of the MAP kinases, which play an important role in cell proliferation and apoptosis [8]. Mitogen/extracellular signal regulated kinase kinase-5 (MEK5), a key kinase of the MEK5-ERK5 pathway, in turn specifically phosphorylates and activates extracellular signal-regulated kinase-5 (ERK5) [9], which directly phosphorylates and activates several transcription factors including c-Myc, Sap-1, c-Fos, Fra-1, and myocyte enhancer factor family members [10, 11], eliciting a variety of

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Manganese superoxide dismutase mediates anoikis resistance and tumor metastasis in nasopharyngeal carcinoma

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ABSTRACT

Metastatic cancer cells are able to survive the loss of attachment to the extracellular matrix (ECM) by developing resistance to anoikis, a specialized form of apoptosis. Here we investigated resistance to anoikis in nasopharyngeal carcinoma cells (NPC). When detached in culture, the highly metastatic S18 NPC cell line exhibited strong resistance to anoikis, as compared to the poorly metastatic S26 NPC cell line. With loss of attachment, S18 cells had lower levels of reactive oxygen species (ROS) and higher levels of manganese superoxide dismutase (MnSOD), an essential mitochondrial antioxidant enzyme. MnSOD knockdown increased the levels of ROS and diminished resistance to anoikis in S18 cells. Conversely, removal of reactive oxygen species (ROS) using NAC or overexpression of MnSOD in S26 cells induced resistance to anoikis. Blocking β-catenin through RNA interference down-regulated MnSOD expression and enhanced anoikis in S18 cells, while β-catenin overexpression enhanced MnSOD expression and suppressed anoikis in S26 cells. In addition, knockdown of MnSOD in S18 cells reduced colony formation *in vitro* and ameliorated lung metastasis *in vivo*. In patients with NPC, MnSOD expression was positively correlated with pathologic tumor stages and negatively correlated with overall survival. These results establish MnSOD as a key mediator of anoikis resistance and tumor metastasis and suggest that β-catenin/MnSOD could be a therapeutic target in NPC.

INTRODUCTION

Nasopharyngeal carcinoma (NPC), derived from the epithelial lining of the nasopharrynx, is a commonly occurring cancer with the highest incidence of metastasis among head and neck cancers in southern China and Southeast Asia [1–4]. Because of its high radio-sensitivity, radiotherapy has been the main strategy for treatment of NPC. However, distant relapse remains the major cause of treatment failure in NPC [5], and the molecular mechanisms underlying NPC metastasis are poorly understood. In order to provide a basis for the development of novel therapeutics for NPC, it is crucial to obtain a better understanding of the molecular mechanisms used by cancer cells to facilitate their survival during metastasis.

While there are many abnormal features of metastatic cancer cells, resistance to anoikis is particularly interesting as it enables cell survival with loss of attachment to the extracellular matrix (ECM) [6, 7]. Metastatic cancer cells develop anoikis resistance by

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Macrophage-mediated inflammatory response decreases mycobacterial survival in mouse MSCs by augmenting NO production

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Mycobacterium tuberculosis **(MTB) is a hard-to-eradicate intracellular microbe, which escapes host immune attack during latent infection. Recent studies reveal that mesenchymal stem cells (MSCs) provide a protective niche for MTB to maintain latency. However, the regulation of mycobacterial residency in MSCs in the infectious microenvironment remains largely unknown. Here, we found that macrophage-mediated inflammatory response during MTB infection facilitated the clearance of bacilli residing in mouse MSCs. Higher inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production were observed in mouse MSCs under macrophage-mediated inflammatory circumstance. Blocking NO production in MSCs increased the survival of intracellular mycobacteria, indicating NO-mediated antimycobacterial activity. Moreover, both nuclear factor κB (NF-κB) and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways were involved in iNOS expression and NO production in inflammatory microenvironment. Furthermore, pro-inflammatory cytokine interleukin-1β could trigger NO production in MSCs and exert anti-mycobacterial activity via NF-κB signaling pathway. Neutralization of interleukin-1β in macrophage-mediated inflammatory microenvironment dampened the ability of mouse MSCs to produce NO. Together, our findings demonstrated that macrophage-mediated inflammatory response during mycobacterial infection promotes the clearance of bacilli in mouse MSCs by increasing NO production, which may provide a better understanding of latent MTB infection.**

Mycobacterium tuberculosis (MTB) infects approximately one-third of the global population and causes tuberculosis (TB), which is the leading bacterial cause of death worldwide[1]. MTB is an intracellular pathogen that can invade and survive within host macrophages[2]. Macrophages express several germ-line encoded pattern-recognition receptors such as Toll-like receptors (TLRs), which recognize conserved pathogen-associated molecular patterns (PAMPs) of MTB. Upon detection of invading MTB, macrophages initiate innate immune response and produce pro-inflammatory cytokines, such as interleukin-1β (IL-1β). In addition, activation of TLRs triggers direct antimicrobial mechanisms[3], such as induction of autophagy[4], production of antimicrobial peptide[5] and generation of nitric oxide (NO)[6–8], all of which play critical roles in the clearance of mycobacteria.

In mouse models of TB infection, the antimicrobial role of NO in host defense is well characterized[9,10]. Lipoproteins from MTB induce TLR-dependent NO production, which kills mycobacteria in macrophages *in vitro*[6,7]. NO production in macrophages requires inducible nitric oxide synthase (iNOS) expression, which could be regulated by PAMPs, pro-inflammatory cytokines (e.g. IL-1β) and interferons (IFNs)[11]. PAMPs and

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Long noncoding RNA NRON contributes to HIV-1 latency by specifically inducing tat protein degradation

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Long noncoding RNAs (lncRNAs) play multiple key regulatory roles in various cellular pathways. However, their functions in HIV-1 latent infection remain largely unknown. Here we show that a lncRNA named NRON, which is highly expressed in resting $CD4^+$ T lymphocytes, could be involved in HIV-1 latency by specifically inducing Tat protein degradation. Our results suggest that NRON lncRNA potently suppresses the viral transcription by decreasing the cellular abundance of viral transactivator protein Tat. NRON directly links Tat to the ubiquitin/proteasome components including CUL4B and PSMD11, thus facilitating Tat degradation. Depletion of NRON, especially in combination with a histone deacetylase (HDAC) inhibitor, significantly reactivates the viral production from the HIV-1-latently infected primary $CD4^+$ T lymphocytes. Our data indicate that lncRNAs play a role in HIV-1 latency and their manipulation could be a novel approach for developing latency-reversing agents.

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REVIEW

Liver fluke infection and cholangiocarcinoma: a review

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Abstract Parasites are significant groups for carcinogenesis among which liver flukes, including *Opisthorchis viverrini* and *Clonorchis sinensis*, are typical representatives causing cholangiocarcinoma (CCA), the second most common primary hepatic malignancy with dismal prognosis. *O. viverrini* is prevalent in Southeast Asia, infecting 10 million people while *C. sinensis* has a wider distribution in East Asia and several Southeast Asian countries, affecting more than 35 million people's health. These two worms have some common characteristics and/or discrepancies in life cycle, genome, and transcriptome. As hot spots in recent years, genome and transcriptome research has extracted numerous novel fluke worm-derived proteins, which are excellent for carcinogenic exploration. However, just a handful of these studies have focused on the metabolic pathway. In this study, the main mechanisms of carcinogenesis of both worms, in terms of mechanical damage, metabolic products and immunopathology, and other possible pathways, will be discussed in detail. This review retrospectively describes the main traits of *C. sinensis* and *O. viverrini*, their molecular biology and core carcinogenic mechanisms in a contrast pattern.

Keywords *Clonorchis sinensis* · *Opisthorchis viverrini* · Cholangiocarcinoma · Omics · Carcinogenic mechanism

Introduction

Parasites are infamous infectious sources for humans and other mammals. In particular, the infection of helminths caused by *Opisthorchis viverrini* and *Clonorchis sinensis* remains a major public health problem in the Southeast and East Asia. This kind of infection leads to chronic inflammation in the host's biliary tract or even more severe problems (Silakit et al. 2015). Epidemiological and experimental evidence strongly indicate that *C. sinensis* and *O. viverrini* are the etiological agents of cholangiocarcinoma (CCA) (Yothaisong et al. 2015). CCA, the second largest contributor to primary liver cancer, is a devastating cancer arising from bile duct epithelial cells; CCA has been characterized as having very poor prognosis and poor response to current therapies (Mihalache et al. 2010; Sithithaworn et al. 2014). In order to gain some insight into these two worms and explore the cause of liver fluke related cancer, we review basic information related to *C. sinensis* and *O. viverrini*, and further discuss genome, and transcriptome research and specific carcinogenic mechanisms.

Characteristics of *O. viverrini* and *C. sinensis*

In 1915, the first *O. viverrini* infection case was reported in Thailand (Leiper 1915). Later, opisthorchias became endemic Southeast Asia countries, including Laos, Cambodia, Thailand, Vietnam, and with some reported cases in Malaysia, Singapore,

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ORIGINAL PAPER

Investigation on oxidative stress of nitric oxide synthase interacting protein from *Clonorchis sinensis*

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Abstract Numerous evidences indicate that excretory–secretory products (ESPs) from liver flukes trigger the generation of free radicals that are associated with the initial pathophysiological responses in host cells. In this study, we first constructed a *Clonorchis sinensis* (*C. sinensis*, *Cs*)-infected BALB/c mouse model and examined relative results respectively at 3, 5, 7, and 9 weeks postinfection (p.i.). Quantitative reverse transcription (RT)-PCR indicated that the transcriptional level of both endothelial nitric oxide synthase (eNOS) and superoxide dismutase (SOD) gradually decreased with lastingness of infection, while the transcriptional level of inducible NOS (iNOS) significantly increased. The level of malondialdehyde (MDA) in sera of infected mouse significantly increased versus the healthy control group. These results showed that the liver of *C. sinensis*-infected mouse was in a state with elevated levels of oxidation stress. Previously, *C. sinensis* NOS interacting protein coding gene (named *Cs*NOSIP) has been isolated and recombinant *Cs*NOSIP (r*Cs*NOSIP) has been expressed in *Escherichia coli*, which has been confirmed to be a component present in *Cs*ESPs and confirmed to play important roles in immune regulation of the host. In the present paper, we investigated the effects of r*Cs*NOSIP on the lipopolysaccharide (LPS)-induced activated RAW264.7, a murine macrophage cell line. We found that endotoxin-free r*Cs*NOSIP significantly promoted the levels of nitric oxide (NO) and reactive oxygen species (ROS) after pretreated with r*Cs*NOSIP, while the level of SOD decreased. Furthermore, r*Cs*NOSIP could also increase the level of lipid peroxidation MDA. Taken together, these results suggested that *Cs*NOSIP was a key molecule which was involved in the production of nitric oxide (NO) and its reactive intermediates, and played an important role in oxidative stress during *C. sinensis* infection.

Keywords *Clonorchis sinensis* · Nitric oxide interacting protein · Oxidative stress · RAW264.7 cells

Introduction

Pathogenic helminths of the genus *Clonorchis sinensis* (*C. sinensis*) infect an estimated 35 million people worldwide (Zhou et al. 2013; Lun et al. 2005; Bae et al. 2013). Clonorchiasis is caused by taking raw or undercooked freshwater fish containing infectious metacercariae (Lv et al. 2011; Wang et al. 2012; Rim et al. 2005). Because of the high correlation between clonorchiasis and cholangiocarcinoma, *C. sinensis* infection has been considered as one of the most important biological agents of cholangiocarcinoma (Huang et al. 2012; Young et al. 2010; Bouvard et al. 2009). Similar to other parasitic helminths, *C. sinensis* continuously releases excretory–secretory products (ESPs) into its extracellular surroundings during infection; ESPs play pivotal roles in host–parasite interactions and can induce inflammatory response of the host. During chronic inflammation, free radical produced

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PLOS | ONE

RESEARCH ARTICLE

Inhibition of *TREM-1* and *Dectin-1* Alleviates the Severity of Fungal Keratitis by Modulating Innate Immune Responses

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Abstract

Purpose

To explore the possibility that inhibiting triggering receptor expressed on myeloid cells-1 (TREM-1) and Dendritic cell-associated C-type lectin-1(Dectin-1) could modulate the innate immune response and alleviate the severity of corneal fungal keratitis.

Method

TREM-1 and Dectin-1 expression was detected in fungus-infected human corneal specimens by real-time PCR. C57BL/6 (B6) mice were injected with *Aspergillus fumigatus* and divided into 4 groups that received subconjunctival injections of PBS and IgG as a control (group I), mTREM-1/IgG fusion protein (group II), the soluble β-glucan antagonist laminarin (group III), or mTREM-1/Fc and laminarin (group IV). Corneal virulence was evaluated based on clinical scores. TREM-1 and Dectin-1 mRNA levels were assayed using real-time PCR. The distribution patterns of TREM-1, Dectin-1 and cellular infiltrates in fungus-infected corneas were examined by immunohistochemistry. Moreover, changes in T Helper Type1 (Th1)-/ T Helper Type1 (Th2)- type cytokines and proinflammatory cytokines were measured.

Results

The expression of TREM-1 and Dectin-1 increased significantly and correlated positively with the progression of fungal keratitis. Most infiltrated cells were neutrophils and secondarily macrophages in infected cornea. The clinical scores decreased after interfering with TREM-1 and Dectin-1 expression in infected mouse corneas. Levels of Th1-type cytokines including interleukin-12 (IL-12), IL-18 and interferon-γ (IFN-γ) were decreased in the cornea, while the levels of Th2-type cytokines, including IL-4, IL-5 and IL-10, showed obvious increases.

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ORIGINAL PAPER

Increased Gal-9 and Tim-3 expressions during liver damage in a murine malarial model

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Abstract Malaria has been one of the most devastating tropical parasite infectious diseases popular around the world. Severe malaria is characterized by multiple organ dysfunctions, especially liver damage. However, the mechanisms of malarial liver injury remain to be better clarified. In this study, Kunming mice inoculated intraperitoneally (i.p.) with 10^6 *Plasmodium berghei* ANKA (*Pb*ANKA)-infected red blood cells (iRBCs) were investigated at days 5, 10, 15, and 20 post-infection (p.i.) to elucidate the profiles of T-cell immunoglobulin and mucin domain-3 (Tim-3) and its ligand galecin-9 (Gal-9) in the development of liver injury. The histopathology of livers and spleens from *Pb*ANKA-infected mice were observed, the parasite burdens of the livers and spleens using quantitative real-time PCR (qRT-PCR), Tim-3- and Gal-9-positive cells in the livers and spleens using immunohistochemical staining, and the mRNA levels of Tim-3, Gal-9, and cytokines in both the livers and spleens using qRT-PCR were examined. Our results showed that parasite burdens in the livers and spleens were significantly increased with time after *Pb*ANKA infection. Histological scores of both the liver and spleen tissues were significantly increased with time; the numbers of Tim-3- and Gal-9-positive cells were significantly increased in both the livers and spleens using immunohistochemical staining, and the mRNA levels of Tim-3 and Gal-9 in the livers and spleens were also significantly increased after infection. Our data suggests that the increase of Tim-3/Gal-9 expressions may play an important role in the liver damage during *P. berghei* infection.

Keywords *Plasmodium berghei* · Liver · Tim-3 · Gal-9 · Mice

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Introduction

Malaria has been one of the most devastating tropical parasite infectious diseases popular around the world. Severe malarial syndromes include severe anemia, hyperparasitemia, cerebral malaria, acute respiratory distress, and clinical jaundice, with the brain, lungs, kidney, and liver all variously affected (WHO 2010). Malaria-associated liver dysfunction is commonly observed in severe malaria, both in adult and pediatric patients (Whitten et al. 2011; Anand and Puri 2005), which is a heterogeneous pathology with variable severity, as symptoms range from mild changes in liver function tests to severe liver failure. Malaria infection can lead to an extremely complicated series of immune response in the liver, and histopathological findings such as portal mononuclear cell infiltration are observed in varying degrees (Murthy et al. 1998). However, the mechanisms behind it are largely unknown.

The T-cell immunoglobulin and mucin domain (Tim) family is a relatively newly described group of molecules with a conserved structure and important immunological functions (Li et al. 2013). It has been reported that after peroral infection of *Toxoplasma gondii*, genetically susceptible C57BL/6 mice develop an unchecked Th1 response associated with the

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Full length article

Immune response induced by oral delivery of *Bacillus subtilis* spores expressing enolase of *Clonorchis sinensis* in grass carps (*Ctenopharyngodon idellus*)

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ABSTRACT

Clonorchiasis, caused by the consumption of raw or undercooked freshwater fish containing infective metacercariae of *Clonorchis sinensis*is (*C.sinensis*), remains a common public health problem. New effective prevention strategies are still urgent to control this food-borne infectious disease. The previous studies suggested *Bacillus subtilis* (*B. subtilis*) spores was an ideal vaccines delivery system, and the *C.sinensis* enolase (*Cs*ENO) was a potential vaccine candidate against clonorchiasis. In the current study, we detected *Cs*ENO-specific IgM levels by ELISA in sera, intestinal mucus and skin mucus in grass carps (*Ctenopharyngodon idella*) through oral administration with *B. subtilis* spores surface expressing *Cs*ENO. In addition, immune-related genes expression was also measured by qRT-PCR. Grass carps orally treated with *B. subtilis* spores or normal forages were used as controls. The results of ELISA manifested that specific IgM levels of grass carps in *Cs*ENO group in sera, intestine mucus and skin mucus almost significantly increased from week 4 post the first oral administration when compared to the two control groups. The levels of specific IgM reached its peak in intestine mucus firstly, then in sera, and last in skin mucus. qRT-PCR results showed that 5 immune-related genes expression had different degree of rising trend in *Cs*ENO group when compared to the two control groups. Our study demonstrated that orally administrated with *B. subtilis* spores expressing *Cs*ENO induced innate and adaptive immunity, systemic and local mucosal immunity, and humoral and cellular immunity. Our work may pave the way to clarify the exact mechanisms of protective efficacy elicited by *B. subtilis* spores expressing *Cs*ENO and provide new ideas for vaccine development against *C. sinensis* infection.

1. Introduction

Clonorchiasis is a serious zoonotic parasitic disease, which is widely prevalent in most Southeast Asian regions such as China, Korea, Vietnam and Russia. It is estimated that approximately 15 million people are infected with this neglected tropical disease globally, of whom nearly 13 million are in China, accounting for more than 85%, and it still presents increment trend [1]. In spite of using many integrated control tactics, it was still hard to eradicate this food-borne parasitosis.

People who infected with *Clonorchis sinensis* (*C. sinensis*) are mainly because of eating raw or undercooked freshwater fish containing infective metacercariae, so cutting off transmission route by interrupting the formation of metacercaria in freshwater fish would be an effective strategy to control clonorchiasis. Vaccine is one of the most effective way to prevent infectious diseases. Studies have shown that fish could be vaccinated by injection, immersion or oral administration. Oral vaccination would be an ideal method for its easy operation, needle-free and feasibility of large-scale promotion.

Our previous studies had found that *C. sinensis* enolase (*Cs*ENO) was the key molecule in the development of metacercaria, and good immune protective efficacy had been obtained by applying it

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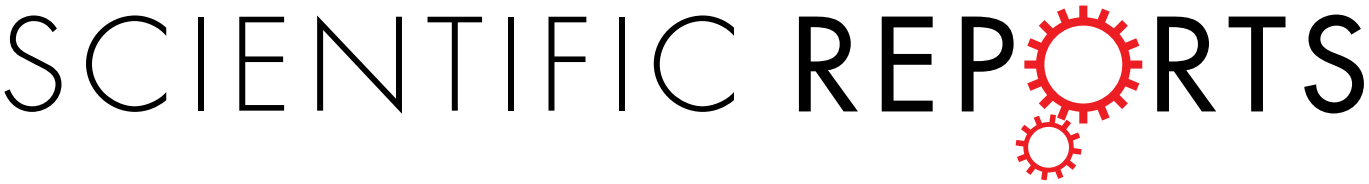

OPEN

IL-33 Contributes to *Schistosoma japonicum*-induced Hepatic Pathology through Induction of M2 Macrophages

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Interleukin (IL)-33 is involved in T helper (Th)2-biased immune responses in mice infected with *Schistosoma*, but the precise mechanism remains to be elucidated. Herein, we investigated the role of IL-33 and its receptor ST2L in hepatic granuloma pathology induced by *Schistosoma japonicum* infection. We found that IL-33 induced the increased production of IL-5 and IL-13 from splenocytes and liver mononuclear cells (MNCs) of infected mice. The infected mice developed significantly higher number of ST2L-expressing cells in spleen and liver. Most of the ST2L-expressing cells in liver were F4/80$^+$ macrophages, indicating the key role of macrophages in the response to IL-33. However, the liver MNCs in male-only worm infection had a poor response to IL-33, though elevated serum IL-33 was observed. ST2L$^+$F4/80$^+$ cells were lower in male-only worm infection than that of mixed infection. IL-33 and soluble egg antigen (SEA) upregulated ST2L expression on macrophages *in vitro* and ST2L-expressing macrophage displayed MHCII$^-$CD11b$^+$M2 phenotype. Macrophage deletion significantly attenuated IL-33-induced type 2 immunity and egg granuloma formation during *S. japonicum* infection. These data demonstrate that IL-33 contributes to hepatic granuloma pathology through induction of M2 macrophages during *S. japonicum* infection.

Fibrosis of the liver is a consequence of chronic liver disease caused by a several factors including infection. The parasitic disease schistosomiasis and resulting hepatitis are often associated with hepatic fibrosis accompanied by significant morbidity and mortality in Asian countries[1,2]. *Schistosoma japonica* is an important cause of hepatic fibrosis in endemic areas of Asia. The fibrosis is thought to be the result of the deposition of large number eggs in the liver of humans by the parasite[3]. The deposited eggs in host liver triggers the formation of granulomata that lead to chronic fibrosis. Thus, better understanding of the mechanistic basis of granuloma formation is important to prevent this infection-associated pathology.

Experimental models of hepatic schistosomiasis clearly demonstrate that host immune responses are essential for the development of granulomatous pathology[4–6]. In *S. japonicum*-infected mice, a T helper (Th)1 response is initiated early in infection followed by an interleukin (IL) 4 and IL-13-mediated dominant Th2 immune response as eggs become lodged in the host liver. At this time macrophages in the egg-induced granuloma are polarized into a M2 macrophage phenotype[7,8]. These macrophages, known as alternatively activated macrophages (AAM), contribute to the development of fibrosis, maintenance of granuloma, tissue repair, and host survival[9,10]. Not surprisingly, in animal models of this infection by male or female *Schistosoma* cercariae alone, the potent Th2-inducing properties are absent since there is no schistosome egg production in single infection.

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The Journal of Immunology

IL-4 Inhibits the Biogenesis of an Epigenetically Suppressive PIWI-Interacting RNA To Upregulate CD1a Molecules on Monocytes/Dendritic Cells

Xue Zhang, Xin He, Chao Liu, Jun Liu, Qifei Hu, Ting Pan, Xiaobing Duan, Bingfeng Liu, Yiwen Zhang, Jingliang Chen, Xingru Ma, Xu Zhang, Haihua Luo and Hui Zhang

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Identification of a Potent and Broad-Spectrum Hepatitis C Virus Fusion Inhibitory Peptide from the E2 Stem Domain

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Xiaojing Chi[1], Yuqiang Niu[1], Min Cheng[1], Xiuying Liu[1], Yetong Feng[2], Fuxiang Zheng[2], Jingjing Fan[1], Xiang Li[1], Qi Jin[1], Jin Zhong[3], Yi-Ping Li[2] & Wei Yang[1]

Hepatitis C virus (HCV) envelope proteins E1 and E2 play an essential role in virus entry. However, the fusion mechanisms of HCV remain largely unclear, hampering the development of efficient fusion inhibitors. Here, we developed two cell-based membrane fusion models that allow for screening a peptide library covering the full-length E1 and E2 amino acid sequences. A peptide from the E2 stem domain, named E27, was found to possess the ability to block E1E2-mediated cell-cell fusion and inhibit cell entry of HCV pseudoparticles and infection of cell culture-derived HCV at nanomolar concentrations. E27 demonstrated broad-spectrum inhibition of the major genotypes 1 to 6. A time-of-addition experiment revealed that E27 predominantly functions in the late steps during HCV entry, without influencing the expression and localization of HCV co-receptors. Moreover, we demonstrated that E27 interfered with hetero-dimerization of ectopically expressed E1E2 in cells, and mutational analysis suggested that E27 might target a conserved region in E1. Taken together, our findings provide a novel candidate as well as a strategy for developing potent and broad-spectrum HCV fusion inhibitors, which may complement the current direct-acting antiviral medications for chronic hepatitis C, and shed light on the mechanism of HCV membrane fusion.

Since its initial identification in 1989, hepatitis C virus (HCV) has been found all over the world, with 7 distinct genotypes and 67 confirmed and 21 unassigned subtypes[1]. Approximately 3% of world's population is infected, making HCV a serious global health problem[2]. Exacerbating the issue, there is currently no vaccine for HCV, and it is estimated that an additional 3–4 million new infections will occur each year[3]. Nonetheless, a large number of compounds have been successfully introduced by combining virological models with high-throughput screening approaches. Although the US Food and Drug Administration recently approved several direct-acting antivirals (DAAs), including Telaprevir, Boceprevir, Sofosbuvir and Viekira Pak, access to these medications is limited by their high cost. Moreover, certain subgroups of difficult-to-treat patients may require adjunctive therapeutic approaches[4,5]. In addition, the drugs that specifically target virus enzymes, such as protease inhibitors, frequently induce resistant mutations. Indeed, evidence shows that the current treatment regimens have resulted in the selection of drug resistant HCV variants[6]; therefore, novel drugs and new strategies are still urgently needed.

HCV is a small, enveloped single-strand RNA virus that belongs to the Hepacivirus genus in the Flaviviridae family. Cell entry by HCV is a multi-step process that begins with attachment of a viral particle to the cell surface via attachment factors, followed by a complex process involving a series of specific cellular entry co-receptors, including scavenger receptor class B type I (SR-BI)[7], tetraspanin CD81[8], claudin-1[9] and occludin[10,11] tight junction proteins. Receptor tyrosine kinases epidermal growth factor receptor, ephrin receptor A2[12], Niemann-Pick C1-like 1 and iron uptake receptor transferrin receptor 1 are also suggested to play roles in HCV entry[13,14]. Envelope protein E1 and E2-mediated interaction of HCV with entry factors leads to internalization of the

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Host Protein Moloney Leukemia Virus 10 (MOV10) Acts as a Restriction Factor of Influenza A Virus by Inhibiting the Nuclear Import of the Viral Nucleoprotein

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ABSTRACT

The viral ribonucleoprotein (vRNP) complex of influenza A viruses (IAVs) contains an RNA-dependent RNA polymerase complex (RdRp) and nucleoprotein (NP) and is the functional unit for viral RNA transcription and replication. The vRNP complex is an important determinant of virus pathogenicity and host adaptation, implying that its function can be affected by host factors. In our study, we identified host protein Moloney leukemia virus 10 (MOV10) as an inhibitor of IAV replication, since depletion of MOV10 resulted in a significant increase in virus yield. MOV10 inhibited the polymerase activity in a minigenome system through RNA-mediated interaction with the NP subunit of vRNP complex. Importantly, we found that the interaction between MOV10 and NP prevented the binding of NP to importin-α, resulting in the retention of NP in the cytoplasm. Both the binding of MOV10 to NP and its inhibitory effect on polymerase activity were independent of its helicase activity. These results suggest that MOV10 acts as an anti-influenza virus factor through specifically inhibiting the nuclear transportation of NP and subsequently inhibiting the function of the vRNP complex.

IMPORTANCE

The interaction between the influenza virus vRNP complex and host factors is a major determinant of viral tropism and pathogenicity. Our study identified MOV10 as a novel host restriction factor for the influenza virus life cycle since it inhibited the viral growth rate. Conversely, importin-α has been shown as a determinant for influenza tropism and a positive regulator for viral polymerase activity in mammalian cells but not in avian cells. MOV10 disrupted the interaction between NP and importin-α, suggesting that MOV10 could also be an important host factor for influenza virus transmission and pathogenicity. Importantly, as an interferon (IFN)-inducible protein, MOV10 exerted a novel mechanism for IFNs to inhibit the replication of influenza viruses. Furthermore, our study potentially provides a new drug design strategy, the use of molecules that mimic the antiviral mechanism of MOV10.

Influenza A viruses (IAVs), which belong to *Orthomyxoviridae* family, cause acute respiratory disease in humans and are responsible for periodic human pandemics as well as seasonal influenza (1–3). The genome of IAV consists of eight negative single-stranded RNA segments, each of which is coiled by multiple nucleoprotein (NP) molecules and associated with one RNA-dependent RNA polymerase (RdRp) complex (4). Three viral proteins—polymerase basic protein 1 (PB1), PB2, and polymerase acidic protein (PA)—constitute a heterotrimeric complex to form the core components of viral polymerase complex (4, 5). The RdRp complex is further associated with NP and viral RNA to form the viral ribonucleoprotein (vRNP) complex, which is the minimal functional unit for mediating viral mRNA transcription and RNA-dependent RNA replication. Especially, the primary transcription is primer dependent, and the primers for mRNA transcription of IAV are obtained via "cap-snatching" of host cellular mRNAs (6–8). During the transcription and replication of IAV, PB1, PB2, and PA are responsible for cap-snatching, transcription initiation, and elongation (9–13). After IAV infection, vRNP complexes are released from endosomes and transported into the nucleus, where viral transcription and replication take place. The vRNP complex utilizes the nuclear localization signals (NLSs) on NP for nuclear import through the cellular importin-α/β-dependent nuclear import pathway (14–17). It has been reported that vRNP complexes are shuttled between the nucleus and cytoplasm during viral replication (18, 19). During the late stage of infection, the newly assembled vRNP complexes are transported out of the nucleus in the guise of nuclear export protein (NEP), matrix protein (M1), and NP (20).

The successful completion of the viral life cycle relies on host factors and processes (21–25). Proper function of vRNPs is key for

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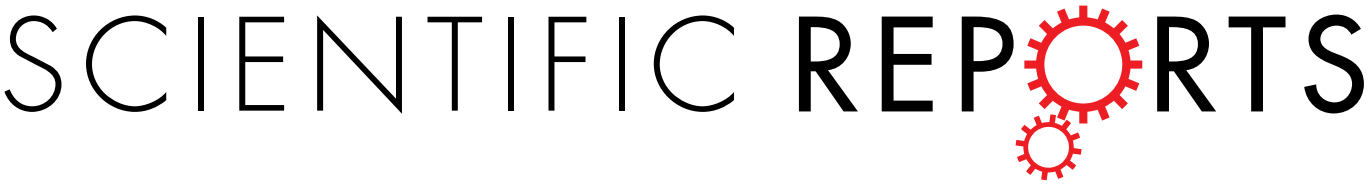

OPEN

HIV-1 Infection-Induced Suppression of the Let-7i/IL-2 Axis Contributes to CD4$^+$ T Cell Death

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The mechanisms underlying HIV-1-mediated CD4$^+$ T cell depletion are highly complicated. Interleukin-2 (IL-2) is a key cytokine that maintains the survival and proliferation of activated CD4$^+$ T cells. IL-2 levels are disturbed during HIV-1 infection, but the underlying mechanism(s) requires further investigation. We have reported that cellular microRNA (miRNA) let-7i upregulates IL-2 expression by targeting the promoter TATA-box region, which functions as a positive regulator. In this study, we found that HIV-1 infection decreases the expression of let-7i in CD4$^+$ T cells by attenuating its promoter activity. The reduced let-7i miRNA expression led to a decline in IL-2 levels. A let-7i mimic increased IL-2 expression and subsequently enhanced the resistance of CD4$^+$ T cells to HIV-1-induced apoptosis. By contrast, the blockage of let-7i with a specific inhibitor resulted in elevated CD4$^+$ T cell apoptosis during HIV-1 infection. Furthermore, by knocking down the expression of IL-2, we found that the let-7i-mediated CD4$^+$ T cell resistance to apoptosis during HIV-1 infection was dependent on IL-2 signaling rather than an alternative CD95-mediated cell-death pathway. Taken together, our findings reveal a novel pathway for HIV-1-induced dysregulation of IL-2 cytokines and depletion of CD4$^+$ T-lymphocytes.

The causes of CD4$^+$ T cell depletion in acquired immunodeficiency syndrome (AIDS) patients have not been fully elucidated. Several predisposing factors have been reported to contribute to HIV-1-induced CD4$^+$ T cell death[1]. For example, viral proteins, including Tat, Nef, Vpr and Env, can induce cell death[2–5]. The integration of proviral DNA into the chromosome is also a trigger of cell death[6]. Recently, Doitsh *et al.* reported that most CD4$^+$ T cell death during HIV-1 infection are caused by caspase-1-mediated pyroptosis triggered by abortive viral infection[7]. They further demonstrated that the incomplete viral transcripts are sensed by interferon-γ-inducible protein 16 (IFI16) and trigger the activation of caspase-1 and pyroptosis[8].

Interleukin-2 (IL-2) is a key cytokine that regulates the proliferation, differentiation and survival of T cells[9]. By promoting the differentiation of T cells into effector T cells, memory T cells and T helper cells following stimulation with an antigen, IL-2 activates immune responses to help the host counteract the invasion of pathogens[10]. IL-2 is mainly secreted by activated CD4$^+$ T cells, and its expression is regulated by a complex network involving transcription factors, chromatin remodeling and CD28 costimulation signaling[11]. It has been reported that HIV-1 infection of CD4$^+$ T cells leads to abnormal expression of the IL-2 gene and disturbs the efficient anti-viral immune responses mediated by IL-2[12–16]. The gradual loss of IL-2 secretion and proliferation is an early sign of T cell exhaustion in HIV-1 infection[17]. IL-2 is also key for maintaining the viability of activated CD4$^+$ T cells by inducing *bcl-2*, *c-myc* and other genes[18,19]. The administration of IL-2 to HIV-1-infected individuals could significantly increase CD4$^+$ T cell counts compared with antiretroviral therapy alone[20–22]. However, the mechanism of dysregulation of IL-2 during HIV-1 infection and its correlation with the depletion of CD4$^+$ T cells have not been properly investigated[23,24].

MicroRNAs represent an important regulator of gene expression in metazoans[25,26]. Most miRNAs downregulate gene expression by suppressing translation or inducing degradation of mRNA via targeting the 3′ UTR[27–29]. In recent years, it has been shown that miRNAs can also activate gene transcription through targeting gene promoter

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REVIEW

History of schistosomiasis epidemiology, current status, and challenges in China: on the road to schistosomiasis elimination

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Abstract Schistosomiasis is a snail-borne disease caused by worms of the genus *Schistosoma*. Worldwide, human schistosomiasis remains a serious public health problem, threatening ~800 million people in 78 countries with a loss of 70 million disability-adjusted life years. *Schistosoma japonicum* is the only human blood fluke that occurs in China. As one of the countries suffering greatly from schistosomiasis, over the past 65 years, China has made great strides in controlling schistosomiasis, blocking the transmission of *S. japonicum* in five provinces, remarkably reducing transmission intensities in the other seven endemic provinces, and China is currently preparing to move toward the elimination of this disease before 2025. However, while on the road to schistosomiasis elimination, emerging challenges merit attention, including severe advanced cases, increased movements of population and livestock, large-area distribution of intermediate host snails, limitations of new drug developments and no vaccine available, as well as imported schistosomiasis and its potential risk.

Lan-Gui Song and Xiao-Ying Wu contributed equally to this work.

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Keywords *Schistosoma japonicum* · Elimination of schistosomiasis · Emerging challenges · P. R. China

Introduction

Human schistosomiasis, as one of the most prevalent neglected tropical diseases, is a snail-borne disease caused by parasitic blood-dwelling flukes, leading to serious socio-economic consequences, only second to malaria, and ~250 million people acquire infection by cercaria-contaminated water contact in 78 countries and ~800 million at risk of this infection, with a loss of 70 million disability-adjusted life years (WHO 2016; The Carter Center 2014; Gray et al. 2010). There are five main species of schistosomes that are able to infect humans, including *Schistosoma japonicum* (Katsurada, 1904), *Schistosoma mansoni* (Sambon, 1907), *Schistosoma haematobium* (Bilharz, 1852), *Schistosoma intercalatum* (Fischer, 1934), and *Schistosoma mekongi* (Voge Bruckner, and Bruce, 1978), among which, *S. japonicum* is the only human blood fluke that occurs in China (WHO 2016). As one of the countries suffering greatly from schistosomiasis, China has made tremendous achievements in schistosomiasis control after six decades of grueling work, despite the extremely severe epidemiologic situation at the start (Utzinger et al. 2005; Xu et al. 2016). Now, China is currently preparing to move toward the elimination of this disease before 2025 (Xu et al. 2016). Here, we summarized the history of schistosomiasis epidemiology in China, the current status of schistosomiasis, and the emerging challenges, including severe advanced patients, increased movements of population and livestock, large-area distribution of intermediate host snails, limitations of new drug developments and no vaccine available, as well as imported schistosomiasis and its potential risk.

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ORIGINAL ARTICLE

High frequency detection of *Toxoplasma gondii* DNA in human neonatal tissue from Libya

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Background: *Toxoplasma gondii* is a parasite that causes significant disease in humans. Toxoplasmosis is normally asymptomatic, unless associated with congenital transmission, or in immunocompromised people. Congenital transmission generally occurs at low frequencies. In this study, we use PCR to investigate possible congenital transmission of *T. gondii* during pregnancy in a cohort of mothers from Libya.

Methods: Two hundred and seventy two pregnant women (producing 276 neonates) were recruited to obtain umbilical cord tissue from their neonates at birth; DNA was extracted from that tissue and tested for *T. gondii* DNA using two specific PCR protocols based on the *sag 1* and *sag 3* genes.

Results: *Toxoplasma gondii* DNA was detected in the umbilical cord DNA from 27 of the 276 neonates giving a prevalence of 9.9% (95% CI 6.8–13.9%). Compared with more commonly reported rates of congenital transmission of 0.1% of live births, this is high. There was no association of infection with unsuccessful pregnancy.

Conclusions: This study shows a high frequency presence of *T. gondii* DNA associated with neonatal tissue at birth in this cohort of 276 neonates from Libya. Although PCR cannot detect living parasites, there is the possibility that this indicates a higher than usual frequency of congenital transmission.

Keywords: Congenital infection, Libya, Miscarriage, Neonatal, PCR, *Toxoplasma gondii*

Introduction

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii* and is an important human and animal disease with a worldwide distribution.[1] The prevalence of infection in the human population is estimated at 30% globally. However prevalence varies with values of 10–20% recorded in the UK, China and US but exceeds 40% in some countries, for example, within South America or continental Europe.[2] Human infection is acquired through contact with oocysts passed in faeces by the definitive host, the cat, or by consuming the tissue cyst(s) found in uncooked or undercooked meat. Congenital transmission, from mother to baby, is also considered a route of transmission – although is considered as rare in humans with occurrence in less than 1 in 1000 live births.[1,3–5] *Toxoplasma* infection is usually asymptomatic. However, in pregnant women a novel infection can cause congenital toxoplasmosis in the foetus, neonate and developing child and some of them may also develop brain diseases such as bipolar disorder and schizophrenia.[4,6–9] Studies on prevalence in pregnant women, for example in China,[10] show that the frequency of infection usually follows the general population prevalence—in this example, averaging 10% in both cases. However, chronic infection of the mother usually does not result in transmission to the foetus and transmission is generally thought to be related to seroconversion during pregnancy[5] or possibly, under some circumstances, reinfection of a chronic infection.[11]

In humans, an estimated 10% of prenatally acquired infections result in abortion (i.e., spontaneous miscarriage) or neonatal death.[12,13] Furthermore, an additional 10–23% of infected newborns will show signs and symptoms present at birth such as retinochoroiditis, intracranial calcifications and hydrocephalus.[12,14,15] It is these abnormalities that significantly reduce

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Hepatitis C Virus Genotype 1 to 6 Protease Inhibitor Escape Variants: *In Vitro* Selection, Fitness, and Resistance Patterns in the Context of the Infectious Viral Life Cycle

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Hepatitis C virus (HCV) NS3 protease inhibitors (PIs) are important components of novel HCV therapy regimens. Studies of PI resistance initially focused on genotype 1. Therefore, knowledge about the determinants of PI resistance for the highly prevalent genotypes 2 to 6 remains limited. Using Huh7.5 cell culture-infectious HCV recombinants with genotype 1 to 6 NS3 protease, we identified protease positions 54, 155, and 156 as hot spots for the selection of resistance substitutions under treatment with the first licensed PIs, telaprevir and boceprevir. Treatment of a genotype 2 isolate with the newer PIs vaniprevir, faldaprevir, simeprevir, grazoprevir, paritaprevir, and deldeprevir identified positions 156 and 168 as hot spots for resistance; the Y56H substitution emerged for three newer PIs. Substitution selection also depended on the specific recombinant. The substitutions identified conferred cross-resistance to several PIs; however, most substitutions selected under telaprevir or boceprevir treatment conferred less resistance to certain newer PIs. In a single-cycle production assay, across genotypes, PI treatment primarily decreased viral replication, which was rescued by PI resistance substitutions. The substitutions identified resulted in differential effects on viral fitness, depending on the original recombinant and the substitution. Across genotypes, fitness impairment induced by resistance substitutions was due primarily to decreased replication. Most combinations of substitutions that were identified increased resistance or fitness. Combinations of resistance substitutions with fitness-compensating substitutions either rescued replication or compensated for decreased replication by increasing assembly. This comprehensive study provides insight into the selection patterns and effects of PI resistance substitutions for HCV genotypes 1 to 6 in the context of the infectious viral life cycle, which is of interest for clinical and virological HCV research.

With more than 100 million chronic infections causing approximately 500,000 deaths annually, hepatitis C virus (HCV) is a major global health and economic burden (1, 2). The six epidemiologically important genotypes differ in ~30% of their sequence and in their sensitivity to antiviral regimens (3–6). In Europe, the Americas, Asia, and Australasia, genotypes 1, 2, and 3 are most prevalent. While genotypes 4, 5, and 6 are more restricted to specific geographical regions in Africa and Asia, they account for 20% of global HCV infections and have spread beyond these primary geographical locations (1, 2, 7).

The development of directly acting antivirals (DAAs) has revolutionized HCV therapy. The main components of interferon-free regimens introduced in the clinic are inhibitors of the HCV nonstructural (NS) proteins NS3 protease (NS3P), NS5A, and NS5B (4–6, 8, 9). Even though DAA-based therapy regimens could cure most patients in clinical trials, failure rates of 5 to 10% are to be expected in real life, due primarily to the development of DAA resistance (4, 8). Given the large number of HCV-infected individuals who will be treated, DAA resistant HCV variants will be common in the future. Treating patients with DAA resistant variants and avoiding DAA resistance will be aided by understanding the determinants and the molecular virology of resistance. The selection of specific resistance substitutions is thought to depend on several factors such as the specific DAA, the level of resistance conferred by the resistance substitution, the genetic barrier to resistance of the HCV isolate, and the fitness of the resistant variant (4, 8, 10). Of note, additional substitutions might compensate for fitness impairment caused by resistance substitutions (10).

Currently, the NS3 protease inhibitors (PIs) telaprevir, boceprevir, simeprevir, asunaprevir, paritaprevir, vaniprevir, and grazoprevir have been licensed (4–6). The first licensed PIs, telaprevir and boceprevir, have linear structures and covalently bind the NS3P active site. Newer PIs have either linear or macrocyclic structures and do not form covalent bonds (9). However, since all PIs target the NS3P active site, substitutions conferring cross-re-

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Glycopeptide Antibiotics Potently Inhibit Cathepsin L in the Late Endosome/Lysosome and Block the Entry of Ebola Virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)*

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Ebola virus infection can cause severe hemorrhagic fever with a high mortality in humans. The outbreaks of Ebola viruses in 2014 represented the most serious Ebola epidemics in history and greatly threatened public health worldwide. The development of additional effective anti-Ebola therapeutic agents is therefore quite urgent. In this study, via high throughput screening of Food and Drug Administration-approved drugs, we identified that teicoplanin, a glycopeptide antibiotic, potently prevents the entry of Ebola envelope pseudotyped viruses into the cytoplasm. Furthermore, teicoplanin also has an inhibitory effect on transcription- and replication-competent virus-like particles, with an IC_{50} as low as 330 nM. Comparative analysis further demonstrated that teicoplanin is able to block the entry of Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) envelope pseudotyped viruses as well. Teicoplanin derivatives such as dalbavancin, oritavancin, and telavancin can also inhibit the entry of Ebola, MERS, and SARS viruses. Mechanistic studies showed that teicoplanin blocks Ebola virus entry by specifically inhibiting the activity of cathepsin L, opening a novel avenue for the development of additional glycopeptides as potential inhibitors of cathepsin L-dependent viruses. Notably, given that teicoplanin has routinely been used in the clinic with low toxicity, our work provides a promising prospect for the prophylaxis and treatment of Ebola, MERS, and SARS virus infection.

Ebola virus (EBOV)[3] is a filamentous-enveloped, single-stranded, and negative-sense RNA virus, which is taxonomically classified to the Filoviridae (1). To date, five species in the *Ebolavirus* genus have been identified, including *Zaire*, *Sudan*, *Reston*, *Tai Forest*, and *Bundibugyo ebolavirus* (2–6). Ebola virus infection leads to severe viral hemorrhagic fever in humans and non-human primates. In March 2014, outbreaks of Ebola viruses began in Guinea and caused over 28,000 cases of infection and over 11,000 deaths, which posed a severe threat to public health worldwide.

The Ebola virus genome contains seven genes that encode the NP, VP35, VP40, glycoprotein (GP), VP30, VP24, and RNA-dependent RNA polymerase (L) virus proteins. To infect host cells, the GPs of Ebola viruses first bind to attachment molecules such as β1 integrins, DC-SIGNs, L-SIGNs, lectins, TIM-1s, Tyro3 family proteins, heparan sulfates, or folate receptor-α (7–13). Ebola viruses are then internalized by macropinocytosis and subsequently transported through the early and late endosomes and the endo/lysosomes (14–16), where the Ebola virus GPs are cleaved by cathepsin L and subsequently cathepsin B to expose the receptor-binding domains (17). After binding the specific receptor NPC1, Ebola viruses release their genomes into the cytoplasm of the host cells (16, 18).

Anti-EBOV vaccines and drugs are under extensive development. Two promising vaccines, rVSVΔG-EBOV-GP and cAd3-EBOV, have been shown to render non-human primates resistant to Ebola virus infections and are currently in clinical trials (19, 20). In addition, the anti-EBOV monoclonal antibody Zmapp, siRNAs, and other compounds that can inhibit Ebola virus infections have been developed (21–24). Furthermore, several clinically approved drugs were also reported to inhibit Ebola virus infections (25, 26). However, because the IC_{50} values of those drugs were relatively high, more anti-EBOV drugs with potent inhibitory activity are urgently needed. To facilitate

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[3] The abbreviations used are: EBOV, Ebola virus; trVLPs, transcription- and replication-competent virus-like particles; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; VSV, vesicular stomatitis virus; GP, glycoprotein; rh, recombinant human; CTSL, cathepsin L; HOPS complex, homotypic fusion and vacuole protein-sorting complex; Z, benzyloxycarbonyl; *t*-Bu, *t*-butyl; IFITM, interferon-inducible transmembrane protein; FDA, Food and Drug Administration.

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Glucocorticoid Induces Hepatic Steatosis by Inhibiting Activating Transcription Factor 3 (ATF3)/S100A9 Protein Signaling in Granulocytic Myeloid-derived Suppressor Cells*

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Glucocorticoids (GCs) used as inflammation suppressors have harmful side effects, including induction of hepatic steatosis. The underlying mechanisms of GC-promoted dysregulation of lipid metabolism, however, are not fully understood. GCs could facilitate the accumulation of myeloid-derived suppressor cells (MDSC) in the liver of animals, and the potential role of MDSCs in GC-induced hepatic steatosis was therefore investigated in this study. We demonstrated that granulocytic (G)-MDSC accumulation mediated the effects of GCs on the fatty liver, in which activating transcription factor 3 (ATF3)/S100A9 signaling plays an important role. ATF3-deficient mice developed hepatic steatosis and displayed expansion of G-MDSCs in the liver and multiple immune organs, which shared high similarity with the phenotype observed in GC-treated wild-type littermates. Adoptive transfer of GC-induced or ATF3-deficient G-MDSCs promoted lipid accumulation in the liver, whereas depletion of G-MDSCs alleviated these effects. Mechanistic studies showed that in MDSCs, ATF3 was transrepressed by the GC receptor GR through direct binding to the negative GR-response element. S100A9 is the major transcriptional target of ATF3 in G-MDSCs. Silencing S100A9 clearly alleviated G-MDSCs expansion and hepatic steatosis caused by ATF3 deficiency or GC treatment. Our study uncovers an important role of G-MDSCs in GC-induced hepatic steatosis, in which ATF3 may have potential therapeutic implications.

Hepatic steatosis is characterized by abnormal lipid accumulation in hepatocytes, which was caused by the imbalance between lipogenesis, lipid catabolism, and free fatty acid uptake (1). Clinical administration of exogenous glucocorticoids (GCs)[2] to patients, subjected to organ transplantation or suffering from severe inflammation-related diseases, is known to cause side effects, including deregulation of lipid metabolism and hepatic steatosis (2, 3). The mechanisms underlying GC-induced hepatic steatosis are still not fully understood. Recent studies have shown that administration of a synthetic GC dexamethasone (Dex) could cause the expansion of distinctive proinflammatory monocytes; they were $CD11b^{+}Gr\text{-}1^{+}$ and expressed signature molecules of tumor-induced myeloid-derived suppressor cells (MDSCs), both in humans and mice (4). Accumulation of immature myeloid ($CD11b^{+}Ly6C^{+}Ly6G^{-}$) cells was also observed in mice fed a high fat diet (5). These observations indicate a possible relationship between GC-induced lipid metabolism and myeloid cell differentiation.

MDSCs represent a heterogeneous population consisting of immature myeloid cells and different stages of myeloid progenitors in which their full differentiation into mature myeloid cells has been prevented (6, 7). MDSC expansion has been observed under various pathological conditions, including but not limited to cancer inflammation, infections, and trauma (8–10). Functioning through suppression of other immune cells, MDSCs are believed to be one of the key brakes in the immune system (7). The clinical beneficial effect of GCs on the prevention of graft-*versus*-host reaction or excessive inflammation is attributed, at least in part, to immune suppressive function of MDSCs (11, 12).

To determine a potential association between MDSCs and GC-induced hepatic steatosis, we have previously performed gene expression analysis of MDSCs purified from liver of Dex-treated and vehicle control mice. We found that the expression of activating transcription factor 3 (ATF3) was dramatically down-regulated in MDSCs upon Dex treatment. ATF3, a basic leucine zipper transcription factor belonging to the ATF/cyclic AMP-response element-binding family is encoded by an adaptive-response gene, *i.e.* its expression is induced by extracellular

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[2] The abbreviations used are: GC, glucocorticoid; MDSC, myeloid-derived suppressor cells; G-MDSC, glucocorticoid MDSC; M-MDSC, monocytic MDSC; Dex, dexamethasone; ATF3, activating transcription factor 3; RLU, relative luciferase unit; GR, GC receptor; nGRE, negative glucocorticoid-response element; DC, dendritic cell; BM, bone marrow; qRT, quantitative reverse transcription; ROS, reactive oxygen species; iMC, immature myeloid cell; TLR, Toll-like receptor; ConA, concanavalin A; Veh, vehicle.

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Functional analysis of microRNA-122 binding sequences of hepatitis C virus and identification of variants with high resistance against a specific antagomir

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MicroRNA 122 (miR-122) stimulates the replication and translation of hepatitis C virus (HCV) RNA by binding to two adjacent sites, S1 and S2, within the HCV 5′UTR. We demonstrated previously that the miR-122 antagomir miravirsen (SPC3649) suppresses the infection of HCV strain JFH1-based recombinants with HCV genotypes 1–6 5′UTR–NS2 in human hepatoma Huh7.5 cells. However, specific S1 mutations were permitted and conferred virus resistance to miravirsen treatment. Here, using the J6 (genotype 2a) 5′UTR–NS2 JFH1-based recombinant, we performed reverse-genetics analysis of S1 (ACACUCCG, corresponding to miR-122 seed nucleotide positions 8–1), S2 (CACUCC, positions 7–2), and ACCC (positions 1–4) at the 5′ end of the HCV genome (5′E); the CC at positions 2–3 of 5′E is involved in miR-122 binding. We demonstrated that the 5′E required four nucleotides for optimal function, and that G or A at position 3 or combined GA at positions 2–3 of 5′E was permitted. In S1 and S2, several single mutations were allowed at specific positions. A UCC → CGA change at positions 4-3-2 of S1, S2, or both S1 and S2 (S1/S2), as well as a C → G change at position 2 of S1/S2 were permitted. We found that 5′E mutations did not confer virus resistance to miravirsen treatment. However, mutations in S1 and S2 induced virus resistance, and combined S1 and/or S2 mutations conferred higher resistance than single mutations. Identification of miR-122 antagomir resistance-associated mutations will facilitate the study of additional functions of miR-122 in the HCV life cycle and the mechanism of virus escape to host-targeting antiviral approaches.

INTRODUCTION

Hepatitis C virus (HCV) persistently infects over 150 million people worldwide and is a leading cause of end-stage chronic liver diseases, including liver cirrhosis and cancer (Alter & Seeff, 2000; Bukh *et al.*, 1993). HCV belongs to genus *Hepacivirus* in the family *Flaviviridae*, and the HCV genome is a positive-sense ssRNA of ~9.6 kb, with a relatively high heterogeneity among isolates (Gottwein & Bukh, 2008; Moradpour *et al.*, 2007). According to differences in nucleotide and amino acid sequences, HCV isolates are classified into seven major genotypes differing by ~30 % in their genome sequences, and subclassified into numerous subtypes with sequence heterogeneity of 15–20 % (Smith *et al.*, 2014). The virus exists as a heterogeneous quasispecies in infected individuals with implications for escape from host immune responses and antivirals (Farci *et al.*, 1997; Forns *et al.*, 1999). The HCV genome consists of a single ORF flanked by 5′ and 3′ UTRs. The ORF is translated into a single polyprotein, which is processed by viral and cellular proteases into 10 structural and non-structural (NS) proteins (Gottwein & Bukh, 2008; Moradpour *et al.*, 2007). Until recently, efficient HCV infectious culture systems were based on the genotype 2a isolate JFH1 in systems permitting studies *in vitro* and *in vivo* (Bukh, 2012; Bukh & Purcell, 2006; Li *et al.*, 2012a, b, 2014; Lindenbach *et al.*, 2005; Wakita *et al.*, 2005).

The 5′UTR of HCV consists of 339–342 nt and forms four major structured domains (Honda *et al.*, 1999). Domain I

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PLOS ONE

RESEARCH ARTICLE

Finasteride Enhances the Generation of Human Myeloid-Derived Suppressor Cells by Up-Regulating the COX2/PGE_2 Pathway

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Abstract

Myeloid-derived suppressor cells (MDSCs) have been known to be a key factor in the regulation of the immune system under numerous conditions such as tumors, infections, autoimmune diseases, and transplantations. In contrast to the proposed deleterious role of MDSCs in tumors and infections, MDSCs with their suppressive function are now proved to have the beneficial potential of suppressing the autoimmune response and promoting tolerance to transplantation. Therefore, the expansion of MDSCs could be a promising therapeutic strategy for many diseases. In this study, we aimed to identify FDA-approved drugs that could aid in the expansion of functional MDSCs. We performed a high-throughput screening (HTS) of FDA-approved drugs based on the *in vitro* human MDSC-differentiation system and identified finasteride (FIN) to have the best potency to aid the generation of human MDSCs. The FIN-induced MDSCs were quite similar to monocytic MDSCs with regard to their surface phenotype, morphology, immunosuppressive function, and related gene expression. Next, we aimed to determine the mechanism of action of FIN and found that FIN induced the expansion of MDSCs through up-regulation of the COX2/PGE_2 pathway by enhancing the activity of COX2 promoter. In addition, the administration of indomethacin (IND), a COX2 inhibitor, abrogated the effect of FIN. Based on these results, we suggested that FIN could find applications in the future in the expansion of MDSCs. Further development of FIN-like compounds could be a novel strategy for generating functional MDSCs for immunosuppressive therapies in various immune disorder conditions.

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Parasites & Vectors

ERRATUM Open Access

Erratum to: Analysis of the mitochondrial maxicircle of *Trypanosoma lewisi*, a neglected human pathogen

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Unfortunately, after the publication of our work [1], we noticed a typo and a mistake in Fig. 2. In Fig. 2, two genes were both labelled with the same abbreviation (*ND1*); these should have been labelled as *ND1* (between COII and GR3) and *ND2* (between GR3 and ATPase6). In addition, the orientation of ATPase6 should have been the same as Cyb.

A corrected version of Fig. 2 is included below.

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AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

Epstein-Barr Virus BZLF1-Mediated Downregulation of Proinflammatory Factors Is Essential for Optimal Lytic Viral Replication

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ABSTRACT

Elevated secretion of inflammatory factors is associated with latent Epstein-Barr virus (EBV) infection and the pathology of EBV-associated diseases; however, knowledge of the inflammatory response and its biological significance during the lytic EBV cycle remains elusive. Here, we demonstrate that the immediate early transcriptional activator BZLF1 suppresses the proinflammatory factor tumor necrosis factor alpha (TNF-α) by binding to the promoter of TNF-α and preventing NF-κB activation. A BZLF1Δ207-210 mutant with a deletion of 4 amino acids (aa) in the protein-protein binding domain was not able to inhibit the proinflammatory factors TNF-α and gamma interferon (IFN-γ) and reduced viral DNA replication with complete transcriptional activity during EBV lytic gene expression. TNF-α depletion restored the viral replication mediated by BZLF1Δ207-210. Furthermore, a combination of TNF-α- and IFN-γ-neutralizing antibodies recovered BZLF1Δ207-210-mediated viral replication, indicating that BZLF1 attenuates the antiviral response to aid optimal lytic replication primarily through the inhibition of TNF-α and IFN-γ secretion during the lytic cycle. These results suggest that EBV BZLF1 attenuates the proinflammatory responses to facilitate viral replication.

IMPORTANCE

The proinflammatory response is an antiviral and anticancer strategy following the complex inflammatory phenotype. Latent Epstein-Barr virus (EBV) infection strongly correlates with an elevated secretion of inflammatory factors in a variety of severe diseases, while the inflammatory responses during the lytic EBV cycle have not been established. Here, we demonstrate that BZLF1 acts as a transcriptional suppressor of the inflammatory factors TNF-α and IFN-γ and confirm that BZLF1-facilitated escape from the TNF-α and IFN-γ response during the EBV lytic life cycle is required for optimal viral replication. This finding implies that the EBV lytic cycle employs a distinct strategy to evade the antiviral inflammatory response.

Infection by the Epstein-Barr virus (EBV) causes infectious mononucleosis and several malignant cancers, including Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma (NPC), and gastric carcinoma, as well as posttransplant lymphomas (1–5). EBV infection is persistent worldwide, but the frequency of EBV-associated NPC is highest in southern China, while Burkitt's lymphoma is most commonly found in equatorial Africa (2, 3). Although the exact mechanism by which EBV causes tumorigenesis remains to be fully defined, two important cofactors are strongly involved in EBV pathogenesis: genetic susceptibility and local diet. Unique polymorphisms of NPC-associated EBV have been identified in Chinese individuals, indicating the existence of EBV variants with higher pathogenic potential for NPC than that seen in the typical Western strains that cause infectious mononucleosis (6–8).

Latent infection with limited gene expression is the default EBV cycle, whereas the lytic cycle is essential for transmission (1, 9). Lytic replication during primary infection or reactivation from the latent cycle is initiated by the expression of the immediate early (IE) viral transactivators BZLF1 and BRLF1. BZLF1, an EBV-encoded transcription factor of the basic-leucine zipper (b-ZIP) family, activates both viral and cellular genes by binding to BZLF1-responsive elements (ZREs), including several transcription factors and inflammatory factors (10).

Inflammatory mediators have complex roles in cancer and infectious diseases, either limiting or promoting these disorders (11–15). Several proinflammatory factors have been fully characterized in experimental and clinical studies, including tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-1α (IL-1α), and IL-1β. TNF-α serves as an antiviral immune factor operating via two different mechanisms: induction of apoptosis in infected cells and activation of the antiviral response in uninfected cells (16–19). For successful infection and replication, viruses employ multiple strategies to escape or hijack the host defenses, including innate immunity and the inflammatory response (15, 17, 20). The EBV lytic cycle evades the host inflammatory responses through the activity of BZLF1, which inhibits both IFN-γ signaling and tumor necrosis factor receptor 1 (TNFR1) signaling (21–23). BZLF1 suppresses the NF-κB signaling pathway by directly binding the p65 subunit (24, 25), acting as an

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Epidemiology of Superficial Fungal Infections in Guangdong, Southern China: A Retrospective Study from 2004 to 2014

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Abstract Superficial fungal infections are common worldwide; however, the distribution of pathogenic species varies among geographical areas and changes over time. This study aimed to determine the epidemiologic profile of superficial fungal infections during 2004–2014 in Guangzhou, Southern China. Data regarding the superficial mycoses from outpatients and inpatients in our hospital were recorded and analyzed. From the 3367 patients that were enrolled in the study, 3385 samples were collected from skin, hair and nail lesions. Of the 697 positive cultures, dermatophytes were the most prevalent isolates (84.36 %), followed by yeasts (14.92 %) and non-dermatophyte molds (0.72 %). *Trichophyton rubrum* (56.24 %) was the most common dermatophyte isolated from cases of tinea unguium (83.92 %), tinea pedis (71.19 %), tinea cruris (91.66 %), tinea corporis (91.81 %) and tinea manuum (65.00 %). *Trichophyton mentagrophytes* (13.35 %) and *Microsporum canis* (10.19 %) were the predominant species associated with cases of tinea faciei (54.55 %) and tinea capitis (54.13 %), respectively. Yeasts and molds were identified primarily from other cases of superficial fungal infections. In conclusion, when compared to previous studies in the same area, the epidemiology of superficial mycoses in Guangdong did not significantly change from 2004 to 2014. The prevalence of causative agents and the spectrum of superficial fungal infections, particularly tinea caused by dermatophyte infection, are similar to reports from several specific regions in China and Europe, whereas increasing incidences of *Trichophyton mentagrophytes* and *Microsporum canis* occurred in Guangdong, China.

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ORIGINAL ARTICLE

Epidemiologic investigation of a family cluster of imported ZIKV cases in Guangdong, China: probable human-to-human transmission

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Zika virus (ZIKV) is an emerging mosquito-borne flavivirus that can potentially threaten South China. A Chinese family of four returning from Venezuela to China was found to be positive for ZIKV when the youngest son's fever was first detected at an airport immigration inspection. They were isolated temporarily in a local hospital in Enping city, Guangdong province, where their clinical data were recorded and urine and saliva were collected to isolate ZIKV and to obtain viral sequences. All of them except the mother presented mild symptoms of rash and fever. Envelope gene sequences from the father, daughter and son were completely identical. Phylogenetic analysis demonstrated that this strain is similar to several imported strains reported in recent months, which are all clustered into a group isolated from 2015 ZIKA outbreaks in Brazil. Together with the climatic features in Venezuela, New York and Guangdong in February, it can be concluded that our subjects are imported cases from Venezuela. With the same viral sequence being shared between family members, neither direct human-to-human nor vector transmission can be ruled out in this study, but the former seems more likely. Although our subjects had mild illness, epidemiologists and public health officials should be aware of the risk of further expansion of ZIKV transmission by local competent vectors.
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Keywords: arbovirus; flavivirus; molecular epidemiology; Guangdong; Zika virus

INTRODUCTION

Although Zika virus (ZIKV) was first identified early in 1947 in Uganda, Africa, outbreaks in French Polynesia in 2013 significantly accelerated the spread of this virus to other parts of the world. ZIKV is a reemerging mosquito-borne flavivirus circulating in a wide range of regions including Africa, South America, and Asia.[1] ZIKV infection can cause serious damage to the central nervous system, such as infant microcephaly and Guillain–Barré Syndrome.[2–4] The virus has proven to be neurotropic in animals, and a recent experiment *in vitro* also showed that it can infect human neural progenitor cells derived from induced pluripotent stem cells.[5] Another study showed that human dermal fibroblasts, epidermal keratinocytes and immature dendritic cells are also permissive to the most recent ZIKV isolates.[6] An animal model of ZIKV infection has been established in AG129 mice by foot pad injection.[7]

By far, *Aedes aegypti* is considered the principal transmission vector of ZIKV,[8] although *Aedes albopictus*, which caused several outbreaks of dengue fever in Guangdong Province of South China in the last two decades, may play a role in the spread of this virus because *A. albopictus* may be a competent vector.[9] There are over 180 000 Chinese in Venezuela, which is one of the regions most heavily affected by ZIKV infection in South America.[10] With frequent people shuttling between South America and Guangdong, there is a potential risk of spreading ZIKV to South China, where *A. albopictus* are active in densely populated communities.

In this study, a family of four flying from Venezuela to Guangzhou of Guangdong Province was found to be ZIKV positive in their peripheral blood. To gain a better understanding of transmission among communities, the phylogenetic relationship between the isolates from this family and others from diverse regions of the world was analyzed. Because this virus may be transmitted directly by body fluids,[11–13] it was also necessary to explore this possibility in this family.

MATERIALS AND METHODS

Infected individuals, samples collection and ethic statements

Four hospitalized individuals from a family (father, mother, daughter and son) were diagnosed with ZIKV infection at Enping People's Hospital. This family had lived in Venezuela for more than two months before 20 February 2016; then they flew to New York

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AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

LETTER TO THE EDITOR

Emergence of the Plasmid-Mediated *mcr-1* Gene in Colistin-Resistant *Enterobacter aerogenes* and *Enterobacter cloacae*

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The gene *mcr-1* was reported as the first plasmid-mediated colistin resistance gene in *Escherichia coli* isolates from food animals, food, and patients in China (1). Since then, detection of *mcr-1*-positive strains has been reported in *Enterobacteriaceae* worldwide (2–4). The emergence of *mcr-1* has the potential to pose a major therapeutic challenge in the treatment of infections caused by *Enterobacteriaceae*. Here, we report the identification of *mcr-1* in colistin-resistant *Enterobacter aerogenes* and *Enterobacter cloacae* isolates.

E. aerogenes strain GB68 was recovered from the vaginal secretion of a 37-year-old pregnant female in Guangzhou, China, in August 2014. She was admitted to a hospital for cervical cerclage. Ten days after the surgery, she was diagnosed with *E. coli* vaginal infection by vaginal culture. In addition, she had mycoplasma coinfection in the reproductive tract. The vaginal infection resolved after treatment with azithromycin and amoxicillin-clavulanic acid. *E. cloacae* strain GB38 was isolated from the urine of a 70-year-old male with urinary tract infection, who was admitted to the same hospital in September 2014.

The species were identified using the API 20E system. Susceptibility to various antimicrobial agents was tested by the agar dilution method (5). *E. aerogenes* GB68 was resistant to polymyxins, including colistin and polymyxin B with a MIC of 16 μg/ml each, cephalosporin, and ciprofloxacin. *E. cloacae* GB38 was resistant to all agents tested, including colistin and polymyxin B with a MIC of >32 μg/ml each, carbapenems, and tigecycline (Table 1). PCR analyses were performed to identify various resistance genes (6). The *mcr-1* gene was detected, as well as genes $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, *qnrS*, and *aac(6′)-Ib*-cr in both isolates and *armA* in *E. cloacae* GB38 (Table 1).

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TABLE 1 Characteristics of *mcr-1*-harboring *Enterobacter aerogenes* and *Enterobacter cloacae* isolates

Characteristic	*Enterobacter aerogenes* GB68	*E. coli* C600 (transconjugant of *E. aerogenes* GB68)	*Enterobacter cloacae* GB38	*E. coli* C600 (transconjugant of *E. cloacae* GB38)	*E. coli* C600
Isolation date	August 2014		September 2014		
Inpatient or outpatient	Inpatient		Inpatient		
Isolation site	Vaginal secretion		Urine		
Resistance gene(s)	*mcr-1*, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, *qnrS*, *aac(6′)-Ib*-cr	*mcr-1*, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$	*mcr-1*, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, *armA*, *qnrS*, *aac(6′)-Ib*-cr	*mcr-1*	
MIC (μg/ml)					
Colistin	16	16	>32	16	<0.25
Polymyxin B	16	16	>32	16	<0.25
Tigecycline	2	0.25	4	0.5	0.5
Ampicillin	>256	>256	>256	16	16
Amoxicillin-clavulanic acid	64	4	256	<2	<2
Cefotaxime	256	>256	>256	<1	<1
Ceftazidime	32	32	>256	2	<1
Cefepime	16	16	>256	<0.5	<0.5
Gentamicin	64	4	>256	4	<1
Amikacin	4	4	>256	4	<2
Ertapenem	<0.25	<0.25	>16	<0.25	<0.25
Imipenem	<0.25	<0.25	>16	<0.25	<0.25
Meropenem	<0.25	<0.25	>16	<0.25	<0.25
Fosfomycin	<16	<16	128	<16	<16
Nitrofurantoin	64	32	64	32	<16
Ciprofloxacin	16	0.016	64	0.032	<0.03

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Effects of G6PD activity inhibition on the viability, ROS generation and mechanical properties of cervical cancer cells

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) deficiency has been revealed to be involved in the efficacy to anti-cancer therapy but the mechanism remains unclear. We aimed to investigate the anti-cancer mechanism of G6PD deficiency. In our study, dehydroepiandrosterone (DHEA) and shRNA technology were used for inhibiting the activity of G6PD of cervical cancer cells. Peak Force QNM Atomic Force Microscopy was used to assess the changes of topography and biomechanical properties of cells and detect the effects on living cells in a natural aqueous environment. Flow cytometry was used to detect the apoptosis and reactive oxygen species (ROS) generation. Scanning electron microscopy was used to observe cell morphology. Moreover, a laser scanning confocal microscope was used to observe the alterations in cytoskeleton to explore the involved mechanism. When G6PD was inhibited by DHEA or RNA interference, the abnormal Young's modulus and increased roughness of cell membrane were observed in HeLa cells, as well as the idioblasts. Simultaneously, G6PD deficiency resulted in decreased HeLa cells migration and proliferation ability but increased ROS generation inducing apoptosis. What's more, the inhibition of G6PD activity caused the disorganization of microfilaments and microtubules of cytoskeletons and cell shrinkage. Our results indicated the anti-cervix cancer mechanism of G6PD deficiency may be involved with the decreased cancer cells migration and proliferation ability as a result of abnormal reorganization of cell cytoskeleton and abnormal biomechanical properties caused by the increased ROS. Suppression of G6PD may be a promising strategy in developing novel therapeutic methods for cervical cancer.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD), an X-linked enzyme that catalyses the first rate-limiting step in the pentose phosphate pathway(PPP), has been revealed to be involved in apoptosis, angiogenesis, tumor occurrence and efficacy to anti-cancer therapy [1,2]. Accumulated evidences have shown that G6PD elevated in various tumors, including leukemia [3], melanoma cancer [4], endometrial carcinomas [5], breast cancers [6], and colon cancers [7]. G6PD provides ribose and nicotinamide adenine dinucleotide phosphate (NADPH) that support biosynthesis and antioxidant defense through PPP [8]. Due to the large biosynthetic demands of a rapidly growing cancer and an adaptation to stressful environments, the PPP has been suggested to promote cancer progression and therapy resistance [9]. Given that G6PD plays a critical role in survival, migration, proliferation, and metastasis of cancer cells, development of potent and selective G6PD inhibitors and elucidation of its underlying anti-tumor mechanism would open up a novel way for cancer therapy.

In our study, two kinds of G6PD inhibitors were used to investigate the underlying anti-cancer mechanism. Dehydroepiandrosterone (DHEA),the non-competitive inhibitor of G6PD, is an adrenal steroid hormone with a wide variety of biological effects both in vivo and in vitro [10–13], showing promising potential in the treatment of different types of cancer cells, such as in breast, liver, and cervix [14–16]. The decline of G6PD activity seen with DHEA treatment is not the result of decreased protein expression but caused by binding to the enzyme–coenzyme substrate ternary complexes in mammalian cells [13,17]. In addition, the inhibition of G6PD activity by shRNA technology was also performed to research the effects of G6PD activity on cervical cancer cells.

Abbreviations: G6PD, Glucose-6-phosphate dehydrogenase; PPP, pentose phosphate pathway; AFM, atomic force microscopy; SEM, scanning electron microscopy; LSCM, laser scanning confocal microscope; RNAi, RNA interference; Ra, arithmetic average roughness; Rq, root-mean-square roughness; NADPH, nicotinamide adenine dinucleotide phosphate; DHEA, dehydroepiandrosterone; ROS, reactive oxygen species; RT, room temperature; MFs, microfilaments; MTs, microtubules; DCFDA, 6-carboxy-2′,7′-dichlorofluorescin diacetate.

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1. Introduction

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Development of an Attenuated Tat Protein as a Highly-effective Agent to Specifically Activate HIV-1 Latency

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Although combined antiretroviral therapy (cART) successfully decreases plasma viremia to undetectable levels, the complete eradication of human immunodeficiency virus type 1 (HIV-1) remains impractical because of the existence of a viral reservoir, mainly in resting memory CD4+ T cells. Various cytokines, protein kinase C activators, and histone deacetylase inhibitors (HDACi) have been used as latency-reversing agents (LRAs), but their unacceptable side effects or low efficiencies limit their clinical use. Here, by a mutation accumulation strategy, we generated an attenuated HIV-1 Tat protein named Tat-R5M4, which has significantly reduced cytotoxicity and immunogenicity, yet retaining potent transactivation and membrane-penetration activity. Combined with HDACi, Tat-R5M4 activates highly genetically diverse and replication-competent viruses from resting CD4+ T lymphocytes isolated from HIV-1-infected individuals receiving suppressive cART. Thus, Tat-R5M4 has promising potential as a safe, efficient, and specific LRA in HIV-1 treatment.

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INTRODUCTION

Latent infection of human immunodeficiency virus type 1 (HIV-1) in resting CD4+ T lymphocytes is the major obstacle in virus eradication after HIV-1-infected individuals receive suppressive combined antiretroviral therapy (cART).[1–5] The deficiency of transcriptional factors such as NF-kB or NFAT,[6,7] the condensed chromatin structure, and epigenetic suppression could contribute to maintaining HIV-1 latency.[6–11] The lack of viral regulatory protein Tat also plays an important role.[12] In addition, a cluster of miRNAs including miR-28, miR-125b, miR-150, miR-223, and miR-382, which are enriched in resting CD4+ T lymphocytes, target the 3′-UTR of HIV-1 mRNA to inhibit the translation of viral proteins, are also involved in HIV-1 latency.[13] Recently, the "shock and kill" strategy has been extensively discussed for the elimination of the viral reservoir.[14,15] By driving latent viruses out of their hiding places, latency activators can expose infected cells under immune surveillance and lead to their eradication. However, there is no reliable method to effectively activate HIV-1 latency at present. Many general lymphocyte activators (*e.g.*, anti-CD3 monoclonal antibody, interleukin-2 (IL-2), and IL-7), non-specific transcription activators such as protein kinase C activators (*e.g.*, prostratin and bryostatin-1), and histone deacetylase inhibitors (HDACi) (*e.g.*, valproic acid and suberoylanilide hydroxamic acid (SAHA)) have been used as latency-reversing agents (LRAs) *ex vivo*. Some of them have even been tested in clinical trials.[16–23] Unfortunately, none of them has been proved to effectively decrease the viral reservoir *in vivo*. Apparently, the development of more special and effective agents to activate viral latency is of great significance.

HIV-1 Tat is a 14–15-kDa early-phase protein of viral transcription. The two effective forms of Tat protein are an 86-amino acid (Tat-86) protein and a 101-amino acid (Tat-101) protein with an extra C-terminal domain. Both forms of Tat exist *in vivo*, with the 101-amino acid form being more immunogenic and inducing a stronger immune reaction.[24,25] Tat specifically activates the HIV-1 promoter by interacting with transactivation response elements and recruiting some important transcriptional factors such as the P-TEFb complex, which contains CDK9 and cyclin T1. CDK9 kinase therefore hyper-phosphorylates the C-terminal domain of RNA polymerase II, leading to a significant increase in transcription efficiency. Tat also recruits CBP/P300 and PCAF to promote histone acetylation. In addition, Tat penetrates the cellular membrane easily because it contains a cell-penetrating peptide, which has widely been used to mediate the entry of various proteins from the extracellular space into the cytoplasm.[26,27] However, Tat causes severe cytopathic effects, including apoptosis, and contributes to HIV-1 pathogenesis.[28–31] Because of the specificity and efficiency of Tat to activate HIV-1 transcription and the proven safety of bioactive recombinant Tat protein in several clinical trials for vaccine development, it is possible to develop a mutated Tat protein as a novel HIV-1 latency activator by decreasing its cytotoxicity and immunogenicity.[32–36] In this study, we employed multiple mutations and explored various combinations of mutants and have developed a recombinant mutated Tat protein for the effective activation of HIV-1 latency.

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PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Developing a Time Series Predictive Model for Dengue in Zhongshan, China Based on Weather and Guangzhou Dengue Surveillance Data

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Data Availability Statement: Dengue surveillance and meteorological data of Zhongshan are available from the Zhongshan Center for Disease Control and Prevention, Institutional Data Access/ Ethics Committee (E-mail: ywkcdc@126.com & Tel: +86-0760-88266545), dengue surveillance data of Guangzhou are available from the Guangzhou Center for Disease Control and Prevention, Institutional Data Access/ Ethics Committee (E-mail: ywk@gzcdc.org.cn & Tel: +86-020-36055887), for researchers who meet the criteria for access to confidential data.

Abstract

Background

Dengue is a re-emerging infectious disease of humans, rapidly growing from endemic areas to dengue-free regions due to favorable conditions. In recent decades, Guangzhou has again suffered from several big outbreaks of dengue; as have its neighboring cities. This study aims to examine the impact of dengue epidemics in Guangzhou, China, and to develop a predictive model for Zhongshan based on local weather conditions and Guangzhou dengue surveillance information.

Methods

We obtained weekly dengue case data from 1st January, 2005 to 31st December, 2014 for Guangzhou and Zhongshan city from the Chinese National Disease Surveillance Reporting System. Meteorological data was collected from the Zhongshan Weather Bureau and demographic data was collected from the Zhongshan Statistical Bureau. A negative binomial regression model with a log link function was used to analyze the relationship between weekly dengue cases in Guangzhou and Zhongshan, controlling for meteorological factors. Cross-correlation functions were applied to identify the time lags of the effect of each

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Dengue Virus Subverts Host Innate Immunity by Targeting Adaptor Protein MAVS

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ABSTRACT

Dengue virus (DENV) is the most common mosquito-borne virus infecting humans and is currently a serious global health challenge. To establish infection in its host cells, DENV must subvert the production and/or antiviral effects of interferon (IFN). The aim of this study was to understand the mechanisms by which DENV suppresses IFN production. We determined that DENV NS4A interacts with mitochondrial antiviral signaling protein (MAVS), which was previously found to activate NF-κB and IFN regulatory factor 3 (IRF3), thus inducing type I IFN in the mitochondrion-associated endoplasmic reticulum membranes (MAMs). We further demonstrated that NS4A is associated with the N-terminal CARD-like (CL) domain and the C-terminal transmembrane (TM) domain of MAVS. This association prevented the binding of MAVS to RIG-I, resulting in the repression of RIG-I-induced IRF3 activation and, consequently, the abrogation of IFN production. Collectively, our findings illustrate a new molecular mechanism by which DENV evades the host immune system and suggest new targets for anti-DENV strategies.

IMPORTANCE

Type I interferon (IFN) constitutes the first line of host defense against invading viruses. To successfully establish infection, dengue virus (DENV) must counteract either the production or the function of IFN. The mechanism by which DENV suppresses IFN production is poorly understood and characterized. In this study, we demonstrate that the DENV NS4A protein plays an important role in suppressing interferon production through binding MAVS and disrupting the RIG-I–MAVS interaction in mitochondrion-associated endoplasmic reticulum membranes (MAMs). Our study reveals that MAVS is a novel host target of NS4A and provides a molecular mechanism for DENV evasion of the host innate immune response. These findings have important implications for understanding the pathogenesis of DENV and may provide new insights into using NS4A as a therapeutic and/or prevention target.

Dengue virus (DENV) (family *Flaviviridae*, genus *Flavivirus*) is an enveloped virus with a positive-sense, single-stranded RNA genome that is responsible for dengue fever (DF) and the more severe, life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (1). It has been estimated that 50 million to 100 million people are infected by DENV worldwide each year, and 500,000 patients progress to severe DHF (2). DENV is categorized into four antigenically related but distinct serotypes, designated DENV1, -2, -3, and -4. The DENV genome is ~11 kb in length and encodes a single polyprotein that can be processed co- and posttranslationally by cellular and viral proteases into three structural (C, prM, and E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins in the endoplasmic reticulum (ER) (3).

As an early response to viral infection, mammalian cells produce type I interferons (IFNs), mainly IFN-α and IFN-β, which repress virus replication (4, 5). This early innate antiviral response is initiated by viral recognition through pathogen recognition receptors (PRRs), including Toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). RIG-I, a member of the RLRs, binds viral RNA, which triggers conformational changes that expose CARD domains for subsequent signaling (6). Signaling by RIG-I requires the adaptor protein designated mitochondrial antiviral signaling adaptor (MAVS) (also known as IPS-1/VISA/Cardif) (7–10). MAVS then transduces signals from RIG-I through CARD-CARD domain interactions, which activate IFN regulatory factor 3 (IRF3) and NF-κB through a signaling cascade involving the cytosolic protein kinases TANK-binding kinase 1 (TBK1) and IκB kinase ε (IKKε) (11), consequently leading to type I IFN production.

Previous studies have shown that DENV infection usually leads to low levels of IFN-α and -β, thus suggesting that DENV may inhibit IFN production after DENV infection (12–16). Several research groups have reported that prevention of STAT1 phosphorylation or induction of STAT2 degradation is a mechanism possibly underlying the antagonistic effects of DENV on IFN signaling

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REVIEW

Current status of *Clonorchis sinensis* and clonorchiasis in China

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The oriental liver fluke, *Clonorchis sinensis*, a pathogen causing clonorchiasis, is of major socio-economic importance in East Asia, including China, Korea and Vietnam. This parasite is now recognized as a biocarcinogen strongly linked to cholangiocarcinoma in humans. Here, we describe the status of clonorchiasis in China, where it has been estimated that more than 15 million patients are affected. This paper also summarizes the major advances in the field of clonorchiasis research during last decade, including diagnosis techniques, pathogenesis and genome/transcriptome/proteome studies in the last years. We strongly hope that our work can stimulate the governments of the countries or regions where clonorchiasis is endemic to pay more attention to this disease and establish related guidelines to prevent and control it.

Keywords: Biocarcinogen, China, Cholangiocarcinoma, Foodborne parasite, Hepatobiliary related disease, Neglected tropical disease

Introduction

Clonorchis sinensis, also known as the Chinese liver fluke, belongs to the family Opisthorchiidae, and is one of the most important foodborne parasites, endemic in Eastern Asian countries, including China, Korea, Vietnam, Thailand and the Far East regions of Russia.[1–4] Based on the most recent national survey in China in 2003, it is estimated that more than 12 million people are infected with *C. sinensis*, and the prevalence of this parasite is expanding in this country.[5]

Despite the wide distribution of the parasite, the disease caused by *C. sinensis* infection, called clonorchiasis, usually exhibits only mild symptoms, therefore, the infection is often neglected by physicians, health policy authorities and even patients. Only heavy and chronic infection with *C. sinensis* will lead to various hepatobiliary related diseases.[1] Currently, clonorchiasis is included in control programs within the group of neglected tropical diseases classified by WHO.[6] It is also classified as one of the group 1 biocarcinogens by the International Agency of Cancer Research (IACR).[7] This paper reviews publications within last decade on *C. sinensis* or clonorchiasis to update and summarize scientific developments associated with this fluke and the diseases it causes.

Search strategy and selection criteria

Literature searches were conducted predominantly using the following databases: CNKI (http://www.cnki.com.cn) and PubMed. Relevant articles or book chapters in Chinese or English from 2005 to 2015 were considered. Search terms included were 'ganxichong', 'huazhigaoxichong', '*Clonorchis sinensis*', and 'clonorchiasis'.

Biology of the worm: a brief introduction to the parasite life cycle

The adult *C. sinensis* is a leaf-shaped hermaphroditic trematode, 10–25 mm in length and 3–5 mm in width with anterior oral sucker and a centrally located ventral sucker and genital pore (Figure 1). The life cycle of *C. sinensis* requires three different types of hosts: freshwater snails, freshwater fish and piscivorous mammals including humans.

A range of freshwater snails (order Mesogastropoda) can serve as the first intermediate hosts, e.g., *Parafossarulus* sp., *Alocinma* sp., and *Bithynia* sp. The snail eats *C. sinensis* eggs and miracidia hatch in the gastrointestinal tract of those snails. The miracidium develops into a sporocyst stage where it undergoes asexual reproduction. Each sporocyst develops into 20–50 rediae within around 17 days and each redia can produce nearly 50 cercariae after three weeks. Recent experimental establishment of the life cycle of *C. sinensis* shows that the asexual reproduction only occurs during summer and it may take 95 days for an egg to develop to mature cercariae with water temperature ranging 24–37°C.[8] Finally, thousands of cercariae from each infected snail are released into the water. The motile cercariae swim actively to

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Infectious Diseases of Poverty

SCOPING REVIEW Open Access

Current status and perspectives of *Clonorchis sinensis* and clonorchiasis: epidemiology, pathogenesis, omics, prevention and control

Ze-Li Tang[1,2], Yan Huang[1,2] and Xin-Bing Yu[1,2*]

Abstract

Clonorchiasis, caused by *Clonorchis sinensis* (*C. sinensis*), is an important food-borne parasitic disease and one of the most common zoonoses. Currently, it is estimated that more than 200 million people are at risk of *C. sinensis* infection, and over 15 million are infected worldwide. *C. sinensis* infection is closely related to cholangiocarcinoma (CCA), fibrosis and other human hepatobiliary diseases; thus, clonorchiasis is a serious public health problem in endemic areas. This article reviews the current knowledge regarding the epidemiology, disease burden and treatment of clonorchiasis as well as summarizes the techniques for detecting *C. sinensis* infection in humans and intermediate hosts and vaccine development against clonorchiasis. Newer data regarding the pathogenesis of clonorchiasis and the genome, transcriptome and secretome of *C. sinensis* are collected, thus providing perspectives for future studies. These advances in research will aid the development of innovative strategies for the prevention and control of clonorchiasis.

Keywords: Clonorchiasis, *Clonorchis sinensis*, Diagnosis, Pathogenesis, Omics, Prevention

Multilingual abstracts

Please see Additional file 1 for translations of the abstract into the five official working languages of the United Nations.

Review

Clonorchis sinensis (*C. sinensis*) and clonorchiasis

C. sinensis is a fish-borne trematode. There are three hosts in the life cycle of *C. sinensis* including freshwater snails (the first intermediate hosts), freshwater fish and occasionally shrimps (the second intermediate hosts), and human or carnivorous mammals (the definitive hosts). The life stages of *C. sinensis* include egg (in definitive hosts or water); miracidium, sporocyst, redia, and cercaria (these four stages occur in freshwater snails); metacercaria (in freshwater fish); and adult (in definitive hosts) (Fig. 1) [1, 2]. *Parafossarulus manchouricus* (*P. manchouricus*) is considered the main first intermediate host of *C. sinensis* in Korea, Russia, and Japan [3–6]. *Melanoides tuberculata* (*M. tuberculata*) serves as an important snail host of *C. sinensis* in Vietnam [7, 8]. Up to 10 species (from 3 families) of snails that are suitable for *C. sinensis* have been found in China, including *Parafossarulus striatulus* (*P. striatulus*, synonym *P. manchouricus*), *Parafossarulus sinensis*, *Bithynia fuchsianus* (*B. fuchsianus*), *Parafossarulus anomalospiralis*, *Alocinma longicornis* (*A. longicornis*), *Bithynia misella*, *Semisulcospira cancellata*, *Semisulcospira amurensis*, *M. tuberculata*, and *Assiminea lutea* [9]. Thus, a total of 10 species belonging to 3 families of freshwater snails can serve as first intermediate hosts [3–9], and most of these snails prefer places with a suitable climate and cool and slow-moving water (such as lakes, streams, ponds, marshes, paddy fields and small ditches). *P. striatulus*, *A. longicornis* and *B. fuchsianus* are the main freshwater snails that can be infected. *Pseudorasbora parva* (*P. parva*) is the most

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ORIGINAL ARTICLE – CANCER RESEARCH

Comprehensive gene and microRNA expression profiling reveals a role for miRNAs in the oncogenic roles of SphK1 in papillary thyroid cancer

Weiwei Liang[1] · Zhiwei Xie[2] · Weiling Cui[1] · Yan Guo[1] · Lijuan Xu[1] · Jueheng Wu[3] · Hongyu Guan[1]

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Abstract
Purpose The oncogenic roles of sphingosine kinase 1 (SphK1) in various cancers, including thyroid cancer, have been well demonstrated. However, the microRNAs (miRNAs) associated with the oncogenic roles of SphK1 remain largely unknown.
Methods Global gene and miRNA expression in TPC1-Vector and TPC1-SphK1 cells was analyzed using digital gene expression (DGE) analysis and small RNA-seq, respectively. miRNA–mRNA interactions were explored by microT-CDS, and the predicted networks were visualized using CytoScape®. Cell invasion and migration were assessed by performing Transwell invasion and wound-healing assays. Luciferase reporter and immunoblot assays were used to evaluate the targeting of fibronectin 1 (FN1) by miR-144-3p.
Results In this study, we found that overexpression of SphK1 differentially regulates the expression of 46 miRNAs and 506 mRNAs in papillary thyroid cancer (PTC) TPC1 cells. Combining bioinformatics predictions of mRNA targets with DGE data on mRNA expression allowed us to identify the mRNA targets of deregulated miRNAs. The direct interaction between miR-144-3p and FN1, which mediates the pro-invasive role of SphK1 in PTC cells, was experimentally validated.
Conclusions Our results demonstrated that SphK1 overexpression drives a regulatory network governing miRNA and mRNA expression in PTC cells. We also demonstrated the roles played by miR-144-3p and FN1 in mediating the oncogenic function of SphK1, which enhanced the understanding of the etiology of PTC.

Keywords SphK1 · Papillary thyroid cancer · Invasion · miR-144-3p · FN1

Introduction

Thyroid cancer is the most common endocrine malignancy and is one of the most rapidly growing cancer diagnoses in the world (Jemal et al. 2011). Follicular epithelial cell-derived thyroid cancer is classified into the following three main histological types: papillary thyroid cancer (PTC), follicular thyroid cancer (FTC) and anaplastic thyroid cancer (ATC). PTC is the most common type of thyroid cancer, as the disease accounts for 85–90% of cases (Siegel et al. 2015). In general, most patients with PTC have a favorable prognosis. However, some patients develop extrathyroidal invasion and lymph node metastases, which leads to a poor prognosis. Therefore, a clear understanding of the molecular mechanisms involved in the development and progression of PTC remains necessary for developing new therapeutic targets.

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ORIGINAL ARTICLE

Comprehensive analysis of hospital-based prospective cohort reveals the unique effectiveness and safety for nucleos(t)ide analogues in HBV patients

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Summary

Background: Nucleos(t)ide analogues (NAs) including lamivudine (LAM), telbivudine (LDT), adefovir dipivoxil(ADV), and entecavir (ETV) have been widely used as anti-HBV drugs. We aimed to study the effectiveness and safety of various NAs.

Methods: Two thousand three hundred and eighty patients with chronic hepatitis B (CHB) were enrolled. The rate of virologic response, optimization therapy, and serologic responses were analyzed.

Results: HBV DNA inhibitory capacity was shown to be LAM + ADV ≈ ETV > LDT > LAM > ADV. Virologic breakthrough rate and proportion of optimized treatment were LAM > ADV > LDT > LAM + ADV > ETV. However, virological response rate showed the opposite trend. The selection of anti-virals, HBeAg-negative, and lower HBV DNA levels after one year of anti-viral treatment, are favorable factors for the maintenance of virologic response.

Conclusions: This study's results were consistent with the major clinical guidelines to recommend ETV and TDF as the preferred treatment for CHB patients. LAM could be used for patients

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Research Article

Complement Factor 3 Could Be an Independent Risk Factor for Mortality in Patients with HBV Related Acute-on-Chronic Liver Failure

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The complement is thought to be involved in the pathogenesis of multiple liver disorders. However, its role in patients with HBV related acute-on-chronic liver failure (HBV-ACLF) remains unclear. Serum levels of the third and fourth complement components (C3, C4) and complement function (CH50) were examined in this prospective, observational study. Associations between their expression and disease activity were analyzed. Survival was analyzed by Kaplan-Meier curves. Predictors of clinical outcome were determined by Cox regression analysis. C3, C4, and CH50 levels were significantly lower in HBV-ACLF patients compared to controls. C3, C4, and CH50 levels were negatively correlated with Tbil levels but positively associated with PTA levels. C3 levels were negatively associated with MELD-Na. C3 levels were significantly lower in HBV-ACLF patients who died compared to patients who survived. In a median hospital stay of 39 days, mortality occurred in 41 patients with a progressive increase based on C3 grade ($P = 0.008$). The actuarial probability of developing mortality was significantly higher in patients with low C3 grade compared to those with high C3 grade ($P < 0.001$). Multivariate Cox regression analysis showed that C3 levels were an independent predictor of mortality. Complement played a pathogenic role in HBV-ACLF patients and C3 was an independent predictor of mortality.

1. Introduction

Chronic hepatitis B (CHB) resulting from a variety of hepatic disease processes caused by HBV infection can lead to acute-on-chronic liver failure (HBV-ACLF), which is a severe clinical syndrome characterized by an acute deterioration of liver function with the eventual development of multiple organ failure [1, 2]. A poor understanding of the pathogenesis of HBV-ACLF and lack of effective treatment options result in extremely high mortality rates [1, 2]. There is a growing appreciation that immunity-mediated inflammation plays an important role in the pathogenesis of HBV-ACLF [3]. In particular, different arms of the innate and adaptive immune system make critical contributions to the progression of HBV-ACLF [1]. However, it is not clear if the complement, which is an important bridge between the innate and adaptive immune systems, plays a role in the pathogenesis of HBV-ACLF.

The complement system comprises approximately 30 proteins that are present either as soluble factors or as membrane-associated proteins [4]. The complement can be activated via the classical, lectin, or alternative pathways, resulting in C3 activation and leading to the generation of the membrane attack complex (C5b-9). Complement activation has also been reported to activate multiple immune cells and play an important role in host defense and wound healing by increasing secretion of inflammatory cytokines. However,

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Clostridium butyricum in combination with specific immunotherapy converts antigen-specific B cells to regulatory B cells in asthmatic patients

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The effect of antigen specific immunotherapy (SIT) on asthma is supposed to be improved. Published data indicate that administration of probiotics alleviates allergic diseases. B cells play important roles in the pathogenesis of allergic diseases. This study aims to modulate antigen specific B cell property by the administration of *Clostridium butyrate* (*CB*) in combination with SIT. The results showed that after a 3-month treatment, the total asthma clinical score and serum specific IgE were improved in the patients treated with SIT, which was further improved in those treated with both SIT and *CB*, but not in those treated with *CB* alone. Treatment with SIT and *CB* increased p300 and STAT3 activation, up regulated the IL-10 gene transcription and increased the frequency of peripheral antigen specific B cells. In conclusion, administration with SIT in combination with *CB* converts Der p 1 specific B cells to regulatory B cells in asthma patients allergic to Der p 1. The data suggest a potential therapeutic remedy in the treatment of allergic diseases.

Allergic asthma is an airway disease mediated by antigen specific IgE. The prevalence of allergic asthma is increasing worldwide in the recent decades[1]. The pathogenesis of asthma has not been fully appreciated yet. Current understanding about the pathogenesis of asthma includes that overproduction of allergen specific IgE; the IgE binds the high affinity receptor of IgE on the surface of mast cells to make mast cells sensitized. Re-exposure to specific allergens activate the sensitized mast cells and trigger the mast cells to release allergic mediators to evoke clinical allergic symptoms[2]. Although research in this area advanced rapidly in recent years, the treatment of asthma is still unsatisfactory[3]. Therefore, to invent novel therapeutic remedies for asthma is of great significance.

The antigen specific immunotherapy (SIT) is the only available effective treatment to target the allergic diseases, such as asthma, instead of the symptoms[4]. SIT is to introduce small doses of the specific antigens to the patients via subcutaneous injection or sublingual absorption, including a build-up phase and a maintenance phase. In the build-up phase, increasing doses of allergens are introduced to patients weekly, while in the maintenance phase, a fixed dose of allergen is introduced to patients monthly[4,5]. One of the mechanisms of SIT is to induce antigen specific immune tolerance in the body, including inducing regulatory T cells (Treg) and regulatory B cells (Breg)[6]. The transforming growth factor-β (TGF-β) and interleukin (IL)-10 are the most common cytokines released from the immune regulatory cells[6]. These mediators suppress other immune effector cell activities so as to suppress the allergic symptoms. To date, the mechanism of immune regulatory cells has not been fully appreciated yet.

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ORIGINAL PAPER

Clonorchis sinensis lysophospholipase inhibits TGF-β1-induced expression of pro-fibrogenic genes through attenuating the activations of Smad3, JNK2, and ERK1/2 in hepatic stellate cell line LX-2

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Abstract Liver fibrosis is a wound healing response associated with chronic liver injury. Hepatic stellate cells (HSCs) activation is a key event in the development of liver fibrosis. Since helminths have the ability to live for decades in the host by establishing an adaptive relationship in the interplay with its hosts, we hypothesize that whether *Clonochis sinensis* LysophospholipaseA (*Cs*LysoPLA), a component of excretory/secretory proteins, can attenuate the fibrogenic response by inhibiting activation of LX-2 cells, thereby balancing the pro-fibrotic and anti-fibrotic response during the *Clonochis sinensis* (*C. sinensis*) infection. In the present study, LX-2 cells were stimulated with *Cs*LysoPLA in the presence of TGF-β1, and the expressions of collagen type I (COL1A1), α-smooth muscle actin (α-SMA), and matrix metalloproteinase 2 (MMP2) were decreased. In addition, *Cs*LysoPLA significantly inhibited the proliferation and migration of LX-2 cells stimulated by TGF-β1. Pretreatment of LX-2 cells with *Cs*LysoPLA attenuated the phosphorylation of Smad3 as well as JNK2 and ERK1/2 in response to the stimulation of TGF-β1. For the first time, our results showed an anti-fibrogenic effect of *Cs*LysoPLA by attenuating the response of LX-2 cells to TGF-β1 through inhibiting the activations of Smad3, ERK1/2, and JNK2.

Keywords Liver fibrosis · *Clonochis sinensis* · *Cs*LysoPLA · LX-2 cells

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Introduction

Chronic liver injury caused by viruses and parasite infection, alcohol abuse, metabolic and autoimmune diseases, and the relative chronic activation of the wound healing reaction results in accumulation of extracellular matrix (ECM), destruction of the normal structure, and the alteration of the normal function, finally leading to liver fibrosis and failure (Pinzani and Macias-Barragan 2010; Anthony et al. 2010). Activated HSCs play a pivotal role in the fibrosis process (Yin et al. 2013). In response to chronic liver injury, HSCs are activated by inflammatory stimulus, such as oxidant stress, apoptotic bodies, and cytokines including TGF-β1, platelet derived growth factor (PDGF) released by hepatocytes, Kupffer cells, and sinusoidal endothelium (Lee and Friedman 2011). Upon activation, HSCs transform to myofibroblast-like cells, proliferate, and migrate to the site of injury, characterized by enhanced α-smooth muscle actin (α-SMA), MMP2 and collagen type I (COL1A1) expressions, ECM production, and pro- and anti-inflammatory cytokines production, such as TGF-β1 (Pellicoro et al. 2014). TGF-β1 is considered as a quite potent pro-fibrogenic cytokine in HSCs. TGF-β1 plays a key role in the process of transdifferentiation of HSCs into myofibroblast-like cells (Gressner et al. 2002). When TGF-β1 activity is enhanced, more collagen is secreted by HSCs to be deposited to the site of injury, aggravating fibrosis

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Clonorchis sinensis Co-infection Could Affect the Disease State and Treatment Response of HBV Patients

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

Abstract

Background

Clonorchis sinensis (*C*. *sinensis*) is considered to be an important parasitic zoonosis because it infects approximately 35 million people, while approximately 15 million were distributed in China. Hepatitis B virus (HBV) infection is a major public health issue. Two types of pathogens have the potential to cause human liver disease and eventually hepatocellular carcinoma. Concurrent infection with HBV and *C*. *sinensis* is often observed in some areas where *C*. *sinensis* is endemic. However, whether *C*. *sinensis* could impact HBV infection or vice versa remains unknown.

Principal Findings

Co-infection with *C*. *sinensis* and HBV develops predominantly in males. Co-infected *C*. *sinensis* and HBV patients presented weaker liver function and higher HBV DNA titers. Combination treatment with antiviral and anti-*C*. *sinensis* drugs in co-infected patients could contribute to a reduction in viral load and help with liver function recovery. Excretory-secretory products (ESPs) may, in some ways, increase HBV viral replication *in vitro*. A mixture of ESP and HBV positive sera could induce peripheral blood mononuclear cells (PBMCs) to produce higher level of Th2 cytokines including IL-4, IL-6 and IL-10 compared to HBV alone, it seems that due to presence of ESP, the cytokine production shift towards Th2. *C*. *sinensis*/HBV co-infected patients showed higher serum IL-6 and IL-10 levels and lower serum IFN-γ levels.

Conclusions/Significance

Patients with concomitant *C*. *sinensis* and HBV infection presented weaker liver function and higher HBV DNA copies. In co-infected patients, the efficacy of anti-viral treatment was better in patients who were prescribed with entecavir and praziquantel than entecavir alone.

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Clinical Significance of Myeloid-Derived Suppressor Cells in Human Immunodeficiency Virus-1/ Hepatitis C Virus-coinfected Patients

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Abstract

Myeloid-derived suppressor cells (MDSCs) are known to accumulate during chronic viral infection, including human immunodeficiency virus-1 (HIV-1) and hepatitis C virus (HCV) infection, and play a critical role in suppressing immune responses. However, the role of MDSCs in HIV/HCV coinfection is unclear. Here, we observed a dramatic increase in monocytic MDSCs (M-MDSCs) level in the peripheral blood of HIV/HCV-coinfected patients compared to that of healthy controls; the level of M-MDSCs proportion in coinfection was not higher than that in HIV or HCV monoinfection. Interestingly, we found the M-MDSCs level in coinfected patients correlated well with $CD4^+$ T cell loss ($r = -0.5680$; $P = 0.0058$), HIV-1 load ($r = 0.6011$; $P = 0.0031$), HCV load ($r = 0.6288$; $P = 0.0017$) and activated $CD38^+$ T cells ($r = 0.5139$; $P = 0.0144$). Initiation of highly active antiretroviral therapy considerably reduced both M-MDSCs and $CD8^+CD38^+$-activated T cell proportion in coinfected patients, and they showed a parallel course of decline. Thus, our results suggest that HIV-1 infection and high chronic immune activation may contribute to the expansion of M-MDSCs and accelerate the disease progression in HIV/HCV-coinfected patients.

Introduction

Human immunodeficiency virus-1 (HIV-1) and hepatitis C virus (HCV) infections are two major global epidemics with millions of affected individuals worldwide. Approximately 2–3% of the world population, or 130–170 million persons, are infected with HCV [1], and HCV infection is often prevalent among HIV-positive patients [2]. HIV/HCV-coinfected patients have higher HCV loads and generally experience more rapid progression to fibrosis, end-stage liver disease and death [3, 4]. This is likely due to further loss of T cell functions, high levels of chronic immune activation and regulatory T cells (Tregs) [5–8]. Whether HCV exerts a negative influence on HIV-1 disease progression continues to be controversial [9–11]. However, following the introduction of HIV and HCV therapy, the survival of coinfected patients is clearly prolonged [4].

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid progenitor cells and immature myeloid cells that are prevented from fully differentiating into mature myeloid cells [12–16]. They migrate out of the bone marrow and accumulate in the blood and peripheral lymphoid tissues. These cells exert immunosuppressive function by inhibiting effector T cell responses, inducing Treg cells or through dampening NK cells function. The main mechanisms for their inhibitory effect are production of arginase-1 (Arg-1), release of NO through inducible nitric oxide synthase expression and production of ROS [12, 17]. In mice, MDSCs have a $Gr1^+CD11b^+$ phenotype [12]. Human MDSCs are less well characterized, whereas they usually are $HLA\text{-}DR^{-/low}CD11b^+CD33^+$ [13]. There are two major MDSCs subsets: monocytic (M-MDSCs) and granulocytic (G-MDSCs). For human MDSCs, the M-MDSCs contain $CD14^+$ cells, while G-MDSCs contain $CD14^-CD15^+$ cells [12, 13]. Expansion of MDSCs is associated with a variety of pathological conditions, including cancers, inflammatory disorders and some autoimmune diseases [13, 18–21]. MDSCs level correlates closely with disease progression and clinical staging in some patients with cancer [22]. Immunotherapies targeting MDSCs are expected to become novel strategies against cancer and other diseases [23].

Recent studies have demonstrated that MDSCs expansion plays an important role in the pathogenesis of viral

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Chloroquine inhibits lytic replication of Kaposi's sarcoma-associated herpesvirus by disrupting mTOR and p38-MAPK activation

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p38-MAPK

ABSTRACT

Lytic infection is essential for the persistent infection and pathogenesis of Kaposi's sarcoma-associated herpesvirus (KSHV), and inhibiting KSHV lytic replication may effectively prevent the occurrence of KSHV-related diseases. Chloroquine (CQ), a well-known antimalarial drug and autophagy inhibitor, exerts broad-spectrum antiviral effects and shows anti-cancer therapeutic potential. However, the ability of CQ and its derivatives to control infection of oncogenic γ-herpesvirus remains undefined. Here we reveal that CQ suppresses KSHV lytic gene expression and virion production, and shows cytotoxicity toward KSHV lytically infected B cells at clinically acceptable doses. CQ suppresses mTOR and p38-MAPK pathway activation during KSHV lytic replication but not latent infection. Furthermore, CQ blocks Epstein-Barr virus (EBV) lytic replication via a distinct mechanism that is invoked to block virion production but does not affect viral gene expression. These results suggest that CQ is an effective antiviral drug against KSHV lytic infection. Our findings indicate that CQ treatment should be considered for controlling KSHV-related diseases, particularly for primary use in co-infection of KSHV with malaria.

1. Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma (KS) in untreated AIDS patients and two lymphoproliferative disorders, primary effusion lymphoma and multicentric Castleman's disease, in immunosuppressed patients (Boshoff and Chang, 2001; Ganem, 2010; Martin, 2007). Although healthy individuals show no symptoms when they are infected with KSHV, immunosuppressed patients are at high risk for such KSHV-related diseases. In addition to co-infection with HIV, KSHV infection also frequently occurs with malarial infection (Nalwoga et al., 2015; Nascimento, 2014; Wakeham et al., 2011), resulting in suppressed immune responses and disease aggravation (Haque et al., 2004; Matar et al., 2015).

KSHV infects endothelial cells and lymphocytes, resulting in the conversion of endothelial-spindle cells and lymphocyte disorders, respectively (Boshoff et al., 1995; Ciufo et al., 2001; Dupin et al., 1999; Ganem, 2006; Kikuta et al., 1997). KSHV lytic replication is essential for persistent infection and pathogenesis, serving as a reservoir of infectious virion particles and an inducer of paracrine factors for inflammation and angiogenesis (Ganem, 2010; Grundhoff and Ganem, 2004). Thus, inhibiting viral lytic replication may effectively prevent the incidence of KSHV persistent infection and the occurrence of KSHV-related diseases. Although many anti-herpesvirus drugs are available (Siakallis et al., 2009; Skorenski and Sienczyk, 2014), few have been assessed for treating KSHV-associated diseases.

Chloroquine (CQ) has been used throughout the 20th century as a well-known and efficacious antimalarial drug (Rolain et al., 2007). CQ likely induces an increase of pH in endosomes, lysosomes and Golgi vesicles (Homewood et al., 1972; Rolain et al., 2007). Although several types of viruses are not sensitive to CQ treatment, such as Nipah virus (Freiberg et al., 2010; Pallister et al., 2009), CQ does show broad-spectrum antiviral effects on viruses that require endosome-mediated entry, that replicate in intracellular vacuoles or that express proteins that undergo post-translational modification in these vacuoles (Madrid et al., 2013; Rolain et al., 2007; Savarino, 2011; Savarino et al., 2003, 2006). Additionally, CQ is commonly used as an inhibitor of autophagy and as a promising chemotherapeutic drug for cancers (Kimura et al., 2013; Manic

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Chimeric Antigen Receptor T Cells Guided by the Single-Chain Fv of a Broadly Neutralizing Antibody Specifically and Effectively Eradicate Virus Reactivated from Latency in CD4$^+$ T Lymphocytes Isolated from HIV-1-Infected Individuals Receiving Suppressive Combined Antiretroviral Therapy

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ABSTRACT

Despite the advent of combined antiretroviral therapy (cART), the persistence of viral reservoirs remains a major barrier to curing human immunodeficiency virus type 1 (HIV-1) infection. Recently, the shock and kill strategy, by which such reservoirs are eradicated following reactivation of latent HIV-1 by latency-reversing agents (LRAs), has been extensively practiced. It is important to reestablish virus-specific and reliable immune surveillance to eradicate the reactivated virus-harboring cells. In this report, we attempted to reach this goal by using newly developed chimeric antigen receptor (CAR)-T cell technology. To generate anti-HIV-1 CAR-T cells, we connected the single-chain variable fragment of the broadly neutralizing HIV-1-specific antibody VRC01 to a third-generation CAR moiety as the extracellular and intracellular domains and subsequently transduced this into primary CD8$^+$ T lymphocytes. We demonstrated that the resulting VC-CAR-T cells induced T cell-mediated cytolysis of cells expressing HIV-1 Env proteins and significantly inhibited HIV-1 rebound after removal of antiviral inhibitors in a viral infectivity model in cell culture that mimics the termination of the cART in the clinic. Importantly, the VC-CAR-T cells also effectively induced the cytolysis of LRA-reactivated HIV-1-infected CD4$^+$ T lymphocytes isolated from infected individuals receiving suppressive cART. Our data demonstrate that the special features of genetically engineered CAR-T cells make them a particularly suitable candidate for therapeutic application in efforts to reach a functional HIV cure.

IMPORTANCE

The presence of latently infected cells remains a key obstacle to the development of a functional HIV-1 cure. Reactivation of dormant viruses is possible with latency-reversing agents, but the effectiveness of these compounds and the subsequent immune response require optimization if the eradication of HIV-1-infected cells is to be achieved. Here, we describe the use of a chimeric antigen receptor, comprised of T cell activation domains and a broadly neutralizing antibody, VRC01, targeting HIV-1 to treat the infected cells. T cells expressing this construct exerted specific cytotoxic activity against wild-type HIV-1-infected cells, resulting in a dramatic reduction in viral rebound *in vitro*, and showed persistent effectiveness against reactivated latently infected T lymphocytes from HIV-1 patients receiving combined antiretroviral therapy. The methods used in this study constitute an improvement over existing CD4-based CAR-T technology and offer a promising approach to HIV-1 immunotherapy.

HIV-1 replication can be efficiently suppressed with combined antiretroviral therapy (cART). However, treatment must be maintained throughout the lifetime of the patient, as this virus can persist in a stable latent reservoir, constituting a major barrier to the establishment of an HIV-1 cure. To date, many strategies have been proposed for the eradication of HIV-1 reservoirs (1–3). Recent efforts have focused on the reactivation of HIV-1-harboring cells with special latency-reversing agents (LRAs), which expose the virus-infected cells to the immune system without global T cell activation. This modality is also known as the shock and kill strategy (4–9). It is well known that HIV-1 can quickly acquire mutations to evade immune recognition (10–12). Several studies have indicated that CD8$^+$ T lymphocytes in infected patients on cART lack HIV-1-specific or effective immune responses and cannot completely eliminate latently infected cells, even after successful reactivation (13, 14). Therefore, the reestablishment of potent antiviral immunity is required for an ultimate kill strategy to eradicate viral reservoirs (7).

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IMMUNOLOGY ORIGINAL ARTICLE

Characteristics of *Schistosoma japonicum* infection induced IFN-γ and IL-4 co-expressing plasticity Th cells

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Summary

Schistosoma japonicum infection can induce granulomatous inflammation and cause tissue damage in the mouse liver. The cytokine secretion profile of T helper (Th) cells depends on both the nature of the activating stimulus and the local microenvironment (e.g. cytokines and other soluble factors). In the present study, we found an accumulation of large numbers of IFN-γ^+ IL-4$^+$ CD4$^+$ T cells in mouse livers. This IFN-γ^+ IL-4$^+$ cell population increased from 0·68 ± 0·57% in uninfected mice to 7·05 ± 3·0% by week 4 following infection and to 9·6 ± 5·28% by week 6, before decreasing to 6·3 ± 5·9% by week 8 in CD4 T cells. Moreover, IFN-γ^+ IL-4$^+$ Th cells were also found in mouse spleen and mesenteric lymph nodes 6 weeks after infection. The majority of the IFN-γ^+ IL-4$^+$ Th cells were thought to be related to a state of immune activation, and some were memory T cells. Moreover, we found that these _S. japonicum_ infection-induced IFN-γ^+ IL-4$^+$ cells could express interleukin-2 (IL-2), IL-9, IL-17 and high IL-10 levels at 6 weeks after _S. japonicum_ infection. Taken together, our data suggest the existence of a population of IFN-γ^+ IL-4$^+$ plasticity effector/memory Th cells following _S. japonicum_ infection in C57BL/6 mice.

Keywords: CD4 T cells; cytokines; interferon-γ; interleukin-4; liver; *Schistosoma japonicum*.

Introduction

Murine and human studies have demonstrated that the normal liver contains significant numbers of resident lymphocytes.[1] They are known as intrahepatic immune cells, and they are phenotypically and functionally distinct from those in the peripheral blood and may mature locally.[2] For example, natural killer (NK) cells, NKT cells, $\gamma\delta$T cells and memory T cells are highly enriched in the liver, whereas the proportions of naive T and B lymphocytes have been reported to be relatively small.[3,4] Intrahepatic immune cells are known to perform diverse immunological functions, generating immunological tolerance to a large number of dietary antigens that are received directly from the gastrointestinal tract and, at the same time, respond to blood- and food-borne pathogens with effective immunological defences.[5,6]

Schistosome infection is initiated by cercariae, which burrow into the skin, transform into schistosomula, and then enter the vasculature and migrate to the portal system, where they mature into adult worms.[7] Eggs are released by female parasites, and they begin to lodge in the interlobular portal venules 4–6 weeks after infection. During the infection, the liver is the principal site that is

Abbreviations: APC, allophycocyanin; BFA, Brefeldin A; BSA, Bovine Serum Albumin; DAPI, 4′,6-diamidino-2-phenylindole; DN, Double Negative; ELISA, Enzyme Linked Immunosorbent Assay; FACS, Fluorescence Activating Cell Sorter; FITC, Fluorescein Isothiocyanae; HBSS, Hank's balanced salt solution; IFN, interferon; IL, interleukin; mAb, monoclonal antibody; MLN, Mesenteric lymph node; PE, phycoerythrin; PerCP, Peridinin-Chlorophyll-Protein Complex; PMA, Phorbol-12-myristate-13-acetate; RT-PCR, Reverse Transcription-Polymerase Chain Reaction; *S. japonicum*, *Schistosoma japonicum*; Th, T helper

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Characteristic amino acid changes of influenza A(H1N1)pdm09 virus PA protein enhance A(H7N9) viral polymerase activity

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Abstract Human coinfection with a novel H7N9 influenza virus and the 2009 pandemic A(H1N1) influenza virus, H1N1pdm09, has recently been reported in China. Because reassortment can occur during coinfection, it is necessary to clarify the effects of gene reassortment between these two viruses. Among the viral ribonucleoprotein complex (vRNP) genes, only the PA gene of H1N1pdm09 enhances the avian influenza viral polymerase activity. Based on a phylogenetic analysis, we show a special evolutionary feature of the H1N1pdm09 PA gene, which clustered with those of the novel H7N9 virus and related H9N2 viruses, rather than in the outgroup as the H1N1pdm09 genes do on the phylogenetic trees of other vRNP genes. Using a minigenome system of the novel H7N9 virus, we further demonstrate that replacement of its PA gene significantly enhanced its polymerase activity, whereas replacement of the other vRNP genes reduced its polymerase activity. We also show that the residues of PA evolutionarily conserved between H1N1pdm09 and the novel H7N9 virus are associated with attenuated or neutral polymerase activity. The mutations associated with the increased activity of the novel H7N9 polymerase are characteristic of the H1N1pdm09 gene, and are located almost adjacent to the surface of the PA protein. Our results suggest that the novel H7N9 virus has more effective PB1, PB2, and NP genes than H1N1pdm09, and that H1N1pdm09-like PA mutations enhance the novel H7N9 polymerase function.

Keywords Influenza A virus · H7N9 · Polymerase activity · PA · Mutation · Reassortment

Introduction

The avian-origin influenza A(H7N9) viruses that have caused outbreaks of human infection in China since March 2013 are novel reassortants [1]. The hemagglutinin (HA) and neuraminidase (NA) genes of the novel H7N9 virus originate from the avian A(H7N3) and A(H7N9) viruses, respectively [1, 2]. Other internal viral genes are derived from the A(H9N2) viruses circulating in different regions of China [3, 4]. The first case of human infection with a novel H7N9 virus was laboratory-confirmed in east China, and the virus has since spread to other regions, with 571 confirmed cases of human infection, including 212 deaths, before February 2015 (http://www.who.int/influenza/human_animal_interface/influenza_h7n9/Risk_Assessment/en/).

Community panic concerning these human infections with the novel H7N9 virus has continued because

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Jun Liu and Feng Huang have contributed equally to this work.

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BZLF1 Attenuates Transmission of Inflammatory Paracrine Senescence in Epstein-Barr Virus-Infected Cells by Downregulating Tumor Necrosis Factor Alpha

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ABSTRACT

Recent studies have shown that inflammatory responses trigger and transmit senescence to neighboring cells and activate the senescence-associated secretory phenotype (SASP). Latent Epstein-Barr virus (EBV) infection induces increased secretion of several inflammatory factors, whereas lytic infections evade the antiviral inflammatory response. However, the changes in and roles of the inflammatory microenvironment during the switch between EBV life cycles remain unknown. In the present study, we demonstrate that latent EBV infection in EBV-positive cells triggers the SASP in neighboring epithelial cells. In contrast, lytic EBV infection abolishes this phenotype. BZLF1 attenuates the transmission of paracrine senescence during lytic EBV infection by downregulating tumor necrosis factor alpha (TNF-α) secretion. A mutant BZLF1 protein, BZLF1Δ207-210, that cannot inhibit TNF-α secretion while maintaining viral transcription, fails to block paracrine senescence, whereas a neutralizing antibody against TNF-α is sufficient to restore its inhibition. Furthermore, latent EBV infection induces oxidative stress in neighboring cells, while BZLF1-mediated downregulation of TNF-α reduces reactive oxygen species (ROS) levels in neighboring cells, and ROS scavengers alleviate paracrine senescence. These results suggest that lytic EBV infection attenuates the transmission of inflammatory paracrine senescence through BZLF1 downregulation of TNF-α secretion and alters the inflammatory microenvironment to allow virus propagation and persistence.

IMPORTANCE

The senescence-associated secretory phenotype (SASP), an important tumorigenic process, is triggered and transmitted by inflammatory factors. The different life cycles of Epstein-Barr virus (EBV) infection in EBV-positive cells employ distinct strategies to modulate the inflammatory response and senescence. The elevation of inflammatory factors during latent EBV infection promotes the SASP in uninfected cells. In contrast, during the viral lytic cycle, BZLF1 suppresses the production of TNF-α, resulting in the attenuation of paracrine inflammatory senescence. This finding indicates that EBV evades inflammatory senescence during lytic infection and switches from facilitating tumor-promoting SASP to generating a virus-propagating microenvironment, thereby facilitating viral spread in EBV-associated diseases.

Cellular senescence, an irreversible arrest of the cell cycle with major hallmarks of senescence-associated heterochromatic foci and DNA segments, is induced by genotoxic or oncogenic stress (1, 2). Oncogene-induced senescence (OIS) is triggered by excessive expression of oncogenes or oncogene-induced replicative stress and acts as an efficient barrier against malignancy (3, 4). However, tumors develop ways to evade OIS during early tumorigenesis (5). Interestingly, senescent cells also secrete proinflammatory factors that are important for tumor progression; this phenotype is called the senescence-associated secretory phenotype (SASP) (6). Recent studies have shown that inflammatory responses trigger and transmit cellular senescence to neighboring cells (7–9), indicating that profound cross talk and signal integration occur between senescent cells and the inflammatory microenvironment and that this communication may promote either tumor progression or suppression.

Herpesviruses produce few transcripts during latent infection. In contrast, during lytic infection, transcripts of the entire herpesvirus genome are produced and cellular machinery and multiple signaling pathways are exploited to facilitate replication and spread (10–12). Host defenses against viral infection include the activation of innate immune and inflammatory responses; however, herpesviruses employ multiple strategies and multiple viral products to evade host defenses (13–16). In addition to being involved in antiviral defenses during acute infection, inflammatory factors are also involved in the progression of persistent infection, cancers, and other inflammatory disorders (10, 17–19).

Studies have identified several inflammatory factors involved in infectious diseases caused by Epstein-Barr virus (EBV) infection that are mediated by both lytic and latent viral gene products (20–25). Levels of these inflammatory factors are elevated during EBV infection, and they elicit chronic inflammation, which leads

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SCIENTIFIC REPORTS

OPEN

Blockage of Galectin-receptor Interactions by α-lactose Exacerbates *Plasmodium berghei*-induced Pulmonary Immunopathology

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Malaria-associated acute lung injury (ALI) is a frequent complication of severe malaria that is often caused by "excessive" immune responses. To better understand the mechanism of ALI in malaria infection, here we investigated the roles of galectin (Gal)-1, 3, 8, 9 and the receptors of Gal-9 (Tim-3, CD44, CD137, and PDI) in malaria-induced ALI. We injected alpha (α)-lactose into mice-infected with *Plasmodium berghei* ANKA (*Pb*ANKA) to block galectins and found significantly elevated total proteins in bronchoalveolar lavage fluid, higher parasitemia and tissue parasite burden, and increased numbers of $CD68^+$ alveolar macrophages as well as apoptotic cells in the lungs after blockage. Additionally, mRNA levels of Gal-9, Tim-3, CD44, CD137, and PDI were significantly increased in the lungs at day 5 after infection, and the levels of CD137, IFN-α, IFN-β, IFN-γ, IL-4, and IL-10 in the lungs were also increased after α-lactose treatment. Similarly, the levels of Gal-9, Tim-3, IFN-α, IFN-β, IFN-γ, and IL-10 were all significantly increased in murine peritoneal macrophages co-cultured with *Pb*ANKA-infected red blood cells *in vitro*; but only IFN-α and IFN-β were significantly increased after α-lactose treatment. Our data indicate that Gal-9 interaction with its multiple receptors play an important role in murine malaria-associated ALI.

Malaria is still a major global health problem. Severe malaria, associated with high morbidity and mortality, is characterized by cerebral malaria, severe anemia, acidosis and hypoglycemia, pulmonary edema, and acute kidney injury[1]. Pulmonary edema, which can be caused by infection with *Plasmodium falciparum*, *P. vivax*, or *P. ovale*[2], is featured by acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) and occurs in approximately 20% of severe malaria patients[3]. ALI develops prior to the onset of cerebral malaria symptoms, and malaria-induced ALI/ARDS pathophysiology demonstrates that inflammatory-mediated increased capillary permeability or endothelial damage leads to diffuse alveolar destruction that can continue after parasite clearance[4]. It has been reported that parasite burden and CD36-mediated sequestration in the lung are primary determinants of ALI in experimental murine malaria[5], and significantly higher rate of monocytes and macrophages are detected in placenta with falciparum malaria infections[6]. However, so far few studies have addressed the characteristics of the immunological response of macrophage infiltration during malaria-induced ALI.

Galectins, beta-galactoside-binding animal lectins, are differentially expressed by various immune cells as well as a wide range of other cell types[7]. So far, fifteen members of the galectin family have been identified in

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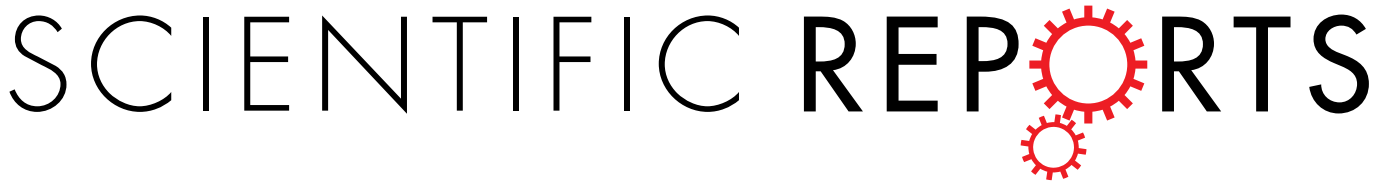

OPEN

Bis-biguanide dihydrochloride inhibits intracellular replication of *M. tuberculosis* and controls infection in mice

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While there is an urgent need to develop new and effective drugs for treatment of tuberculosis (TB) and multi-drug resistant TB (MDR-TB), repurposing FDA (U.S. Food and Drug Administration) -approved drugs for development of anti-TB agents may decrease time and effort from bench to bedside. Here, we employed host cell-based high throughput screening (HTS) assay to screen and characterize FDA-approved, off-patent library drugs for anti-*Mycobacterium tuberculosis* (MTB) activities. The cell-based HTS allowed us to identify an anti-cancer drug of bis-biguanide dihydrochloride (BBD) as potent anti-mycobacteria agent. Further characterization showed that BBD could inhibit intracellular and extracellular growth of *M. smegmatis* and slow-growing *M. bovis* BCG. BBD also potently inhibited replication of clinically-isolated MTB and MDR-TB strains. The proof-of-concept study showed that BBD treatment of MTB-infected mice could significantly decrease CFU counts in the lung and spleen. Notably, comparative evaluation showed that MTB CFU counts in BBD-treated mice were lower than those in rifampicin-treated mice. No apparent BBD side effects were found in BBD-treated mice. Thus, our findings support further studies to develop BBD as a new and effective drug against TB and MDR-TB.

Tuberculosis (TB) remains one of the leading causes of global morbidity and mortality among infectious diseases largely due to HIV (Human Immunodeficiency Virus) pandemics and drug-resistance[1]. Multidrug-resistant TB (MDR-TB) is defined as an infection form of *M. tuberculosis* (MTB) resistant to at least two first-line anti-TB drugs: isoniazid (INH) and rifampicin (RIF)[2]. The 2014 WHO (World Health Organization) Global TB Report estimated that MDR-TB occurs in 3.5% and 20.5% of new and previously treated TB cases, respectively[3] and about 480,000 MDR-TB cases were reported in 2013[3]. MDR-TB is quite difficult to treat, as the treatment is long and expensive, and often associated with high frequencies of adverse events and failure[4]. While MDR-TB poses a significant threat to global TB control[5], there is an urgent need to develop new and effective anti-TB drugs.

Very few of the current anti-TB drugs can exert bactericidal effect on intracellular MTB[6,7]. MTB can survive and replicate in host cells, and exploit cellular shelters to alleviate or even avoid the killing by anti-TB drugs[8,9]. Moreover, the ability of MTB to undergo adaptive metabolic changes within the host may also affect drug activity and potency[10,11]. Rational drug development strategy should target both bacterial and host/cellular factors[12,13] for developing novel drugs capable of inhibiting or killing intracellular MTB. Thus, host cell-based drug screening assay may serve as an ideal model system to select new anti-TB drug candidates against intracellular MTB.

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The Journal of Infectious Diseases

MAJOR ARTICLE

Bioaerosol Sampling in Modern Agriculture: A Novel Approach for Emerging Pathogen Surveillance?

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Background. Modern agricultural practices create environmental conditions conducive to the emergence of novel pathogens. Current surveillance efforts to assess the burden of emerging pathogens in animal production facilities in China are sparse. In Guangdong Province pig farms, we compared bioaerosol surveillance for influenza A virus to surveillance in oral pig secretions and environmental swab specimens.

Methods. During the 2014 summer and fall/winter seasons, we used 3 sampling techniques to study 5 swine farms weekly for influenza A virus. Samples were molecularly tested for influenza A virus, and positive specimens were further characterized with culture. Risk factors for influenza A virus positivity for each sample type were assessed.

Results. Seventy-one of 354 samples (20.1%) were positive for influenza A virus RNA by real-time reverse-transcription polymerase chain reaction analysis. Influenza A virus positivity in bioaerosol samples was a statistically significant predictor for influenza A virus positivity in pig oral secretion and environmental swab samples. Temperature of <20°C was a significant predictor of influenza A virus positivity in bioaerosol samples.

Discussions. Climatic factors and routine animal husbandry practices may increase the risk of human exposure to aerosolized influenza A viruses in swine farms. Data suggest that bioaerosol sampling in pig barns may be a noninvasive and efficient means to conduct surveillance for novel influenza viruses.

Keywords. One Health; bioaerosol; influenza A virus; swine; China; modern agriculture; emerging pathogens.

Modern agricultural production systems produce some of the safest and least expensive meat products the world has ever known. However, the large scale of these farms provides opportunities for some pathogens to be enzootic, which could lead to the emergence and spread of novel pathogens [1–3]. The likelihood of an emergence event occurring in such settings is thought to be particularly high for influenza A viruses [4].

Influenza A viruses are a major cause of morbidity and mortality among human and animal populations worldwide [5, 6]. Numerous studies have been conducted to better understand influenza A viral ecology, particularly conditions that may increase the propensity for influenza A viruses to reassort in animals and cross-over to human populations. There is strong evidence documenting that swine are important for the genetic evolution and potential emergence of novel influenza A viruses [7–12].

To keep up with increasing pork demand, the pork industry is shifting to the modern agricultural practice of rearing pigs in larger, more-efficient concentrated animal feeding operations. This is perhaps most notable in China, which has seen the largest increase in domestic pork production and consumption in the past 10 years. There is concern that such a move to larger production facilities, without increases in biosecurity measures, will create environments more conducive for the mixing and generation of novel pathogens [13], which puts workers and their family members at increased risk of infection.

Given that current surveillance methods to detect zoonotic influenza A virus among swine are invasive and require extensive resources to operate, production managers may be hesitant to adopt them. Additionally, there are economic barriers to the transparent monitoring of swine herds. Alternative methods that embrace a One Health approach and incorporate human, animal, and environmental testing strategies could be a way to overcome these challenges, but few of these methods have been developed and evaluated.

One technology that has potential for the noninvasive detection of influenza A viruses in swine production facilities is bioaerosol sampling. While recent studies of various bioaerosol sampling devices have shown some promise in overcoming the inherent challenges of low detection efficiency for different swine viruses [14–17], bioaerosol sampling data for influenza A

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IMMUNOLOGY ORIGINAL ARTICLE

Atorvastatin promotes the expansion of myeloid-derived suppressor cells and attenuates murine colitis

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Summary

Statins, widely prescribed as cholesterol-lowering drugs, have recently been extensively studied for their pleiotropic effects on immune systems, especially their beneficial effects on autoimmune and inflammatory disorders. However, the mechanism of statin-induced immunosuppression is far from understood. Here, we found that atorvastatin promoted the expansion of myeloid-derived suppressor cells (MDSCs) both *in vitro* and *in vivo*. Atorvastatin-derived MDSCs suppressed T-cell responses by nitric oxide production. Addition of mevalonate, a downstream metabolite of 3-hydroxy-3-methylglutaryl coenzyme A reductase, almost completely abrogated the effect of atorvastatin on MDSCs, indicating that the mevalonate pathway was involved. Along with the amelioration of dextran sodium sulphate (DSS) -induced murine acute and chronic colitis, we observed a higher MDSC level both in spleen and intestine tissue compared with that from DSS control mice. More importantly, transfer of atorvastatin-derived MDSCs attenuated DSS acute colitis and T-cell transfer of chronic colitis. Hence, our data suggest that the expansion of MDSCs induced by statins may exert a beneficial effect on autoimmune diseases. In summary, our study provides a novel potential mechanism for statins-based treatment in inflammatory bowel disease and perhaps other autoimmune diseases.

Keywords: atorvastatin; immunosuppression; murine colitis; myeloid-derived suppressor cells; nitric oxide.

Introduction

Statins are inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme that catalyses the conversion of HMG-CoA to mevalonate in cholesterol synthesis. Statins have been used extensively as cholesterol-lowering agents and for the treatment of cardiovascular disease. However, accumulating evidence has demonstrated that statins have extensive immunomodulatory and anti-inflammatory properties in addition to their essential role in lipid lowering.[1–3]

In recent years, considerable interest has arisen in statin-based therapy of autoimmune and inflammatory disorders.[1,2,4] Studies have found that statin treatment results in an improved clinical outcome in animal models of murine colitis,[5–9] experimental autoimmune encephalomyelitis,[10,11] arthritis[12] and systemic lupus erythematosus.[13] These beneficial effects are partly attributed to statins' immunomodulatory role in suppressing T helper type 1 and type 17 responses, while promoting T helper type 2 responses.[1,2] Atorvastatin (Lipitor), a current extensively prescribed statin drug, has recently gained much attention in inflammatory bowel disease due to its immunomodulating

Abbreviations: CFSE, 5,6 carboxyfluorescein diacetate, succinimidyl ester; CM-H2DCFDA, 5-(and-6)-chloromethyl-2,7-dichlorodihydrofluorescein diacetate, acetyl ester; DSS, dextran sodium sulphate; GM-CSF, granulocyte–macrophage colony-stimulating factor; G-MDSCs, granulocytic MDSCs; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IFN-γ, interferon-γ; IL-17, interleukin-17; iNOS, inducible nitric oxide synthase; JAK/STAT, Janus kinase/singal transducer and activator of transcription; LPMCs, lamina propria mononuclear cells; MDSCs, myeloid-derived suppressor cells; mLN, mesenteric lymph nodes; M-MDSCs, monocytic MDSCs; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase complex; PE, phycoerythrin; PP, Peyer's patches; ROS, reactive oxygen species; Th1, T helper type 1

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RESEARCH ARTICLE

Astrocyte Elevated Gene 1 Interacts with Acetyltransferase p300 and c-Jun To Promote Tumor Aggressiveness

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ABSTRACT Astrocyte elevated gene 1 (AEG-1) is an oncoprotein that strongly promotes the development and progression of cancers. However, the detailed underlying mechanisms through which AEG-1 enhances tumor development and progression remain to be determined. In this study, we identified c-Jun and p300 to be novel interacting partners of AEG-1 in gliomas. AEG-1 promoted c-Jun transcriptional activity by interacting with the c-Jun/p300 complex and inducing c-Jun acetylation. Furthermore, the AEG-1/c-Jun/p300 complex was found to bind the promoter of c-Jun downstream targeted genes, consequently establishing an acetylated chromatin state that favors transcriptional activation. Importantly, AEG-1/p300-mediated c-Jun acetylation resulted in the development of a more aggressive malignant phenotype in gliomas through a drastic increase in glioma cell proliferation and angiogenesis *in vitro* and *in vivo*. Consistently, the AEG-1 expression levels in clinical glioma specimens correlated with the status of c-Jun activation. Taken together, our results suggest that AEG-1 mediates a novel epigenetic mechanism that enhances c-Jun transcriptional activity to induce glioma progression and that AEG-1 might be a novel, potential target for the treatment of gliomas.

KEYWORDS E1A binding protein p300 (p300), acetylation, astrocyte elevated gene 1 (AEG-1), c-Jun transcription factor, glioma

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L.L. and H.G. contributed equally to this article.

Glioma is the most common and aggressive type of central nervous system tumor (1). Despite intensive research and clinical efforts, the prognosis for patients with this tumor type remains poor, largely attributable to its highly invasive and fast proliferating phenotype. The median life expectancy of patients with a grade IV glioma, known as a glioblastoma multiforme (GBM), is less than 1 year (2). Therefore, the definition of appropriate targets against which effective strategies to treat glioma may be developed represents a major goal in glioma research. A better comprehension of the molecular mechanisms mediating glioma progression is crucial to developing an efficacious therapeutic strategy that prevents the infiltration, invasion, and proliferation of glioma cells.

The product of the gene astrocyte elevated gene 1 (*AEG-1*), also known as the metadherin (*MTDH*) or *LYRIC* gene, was initially identified to be a novel protein whose expression is induced by human immunodeficiency virus type 1 (HIV-1) or by tumor necrosis factor alpha (TNF-α) in primary human fetal astrocytes (3–6). AEG-1 is a multifunctional protein that interacts with diverse partners in different types of cancers and promotes the development of essentially all hallmarks of cancer (7–12). Previous

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ORIGINAL PAPER

Assessment of the effect of treatment and assistance program on advanced patients with schistosomiasis japonica in China from 2009 to 2014

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Abstract Schistosomiasis is one of the most important zoonoses, threatening approximately 800 million people in 78 countries with a loss of 70 million disability-adjusted life years. Over the past six decades, China has made remarkable achievements in morbidity control, but disability and mortality control remains much to desire; thus, advanced schistosomiasis is a growing problem when on the road to schistosomiasis elimination. Since 2005, China has initiated a national treatment and assistance program to advanced patients, aiming to improve patients' symptoms and quality of life. Here, we conducted a two-phase study to evaluate the program's implementation and effect on advanced patients from 2009 to 2014 in Jiangxi Province, China. A total of 6425 advanced schistosomiasis cases were included in this study. For those having been treated and assisted (90.7 %), the cure or improvement rate was over 99.9 %, with 668 (11.5 %) cases having reached clinical cure and 5152 (88.4 %) cases' condition having improved, which can be partially reflected in the significant decline of the proportion of hepatomegaly (splenomegaly), the degree of liver fibrosis, ascites-related indicators (abdominal girth and frequency of shifting dullness), and portal hypertension-related indices (inner diameter of portal vein and frequency of subcutaneous varicose vein of abdominal wall). Besides, it was estimated to have saved 2004 years of life lost at total. Therefore, the government should continue support and increase input of treatment and assistance program so that this project can reach more patients, leading to consolidation of achievements of schistosomiasis control and contribution to schistosomiasis elimination.

Keywords *Schistosoma japonicum* · Advanced schistosomiasis · Evaluation · Treatment and assistance · China

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Introduction

Schistosomiasis is one of the most important zoonoses, which does harm to humans' health and socioeconomic developments, threatening approximately 800 million people in 78 countries with a loss of 70 million disability-adjusted life years (WHO 2016; Gray et al. 2010). There are three major species of *Schistosoma*, including *Schistosoma japonicum*, *Schistosoma mansoni*, and *Schistosoma haematobium*, among which, *S. japonicum* is the only human blood fluke that occurs in China (Colley et al. 2014; Richter et al. 2016). As one of the counties highly suffering from schistsomiasis, over the past six decades, China has made remarkable achievements in schistosomiasis control (Xu et al. 2016; Zhou et al. 2005). According to the latest report on endemic status of schistosomiasis in China in 2014, China has disrupted transmission in five provinces (Guangdong, Shanghai, Fujian, Guangxi, and Zhejiang) and dramatically reducing transmission intensities

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Review

Artemisinin and its derivatives in treating protozoan infections beyond malaria

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ABSTRACT

Parasitic protozoan diseases continue to rank among the world's greatest global health problems, which are also common among poor populations. Currently available drugs for treatment present drawbacks, urging the need for more effective, safer, and cheaper drugs. Artemisinin (ART) and its derivatives are some of the most important classes of antimalarial agents originally derived from *Artemisia annua* L. However, besides the outstanding antimalarial and antischistosomal activities, ART and its derivatives also possess activities against other parasitic protozoa. In this paper we review the activities of ART and its derivatives against protozoan parasites in vitro and in vivo, including *Leishmania* spp., *Trypanosoma* spp., *Toxoplasma gondii*, *Neospora caninum*, *Eimeria tenella*, *Acanthamoeba castellanii*, *Naegleria fowleri*, *Cryptosporidium parvum*, *Giardia lamblia*, and *Babesia* spp. We conclude that ART and its derivatives may be good alternatives for treating other non-malarial protozoan infections in developing countries, although more studies are necessary before they can be applied clinically.

Contents

Abbreviations: ART, artemisinin; b.i.d., two times per day; CH_2CL_2, dichloromethane; CI, cell index; DART, dehydroartemisinin; Deoxy-ATS, deoxygenated artesunate; Deoxy-DHA, deoxydihydroartemisinin; DHA, dihydroartemisinin; DMSO, dimethyl sulphoxide; HFF, human foreskin fibroblast; IC_{50}, concentration that causes 50% inhibition of growth; IC_{90}, concentration that causes 90% inhibition of growth; i.g., intragastric administration; i.m., intramuscular injection; i.p., intraperitoneal injection; iTRAQ, isobaric tags for relative and absolute quantitation; i.v., intravenous injection; Luc value, luciferase value; MeOH, methanol; NO, nitric oxide; PCV, packed cell volume; PGDH, phosphoglycerate dehydrogenase; p.i., post-infection; PI staining, propidium iodide staining; p.o., oral administration; PSAT, phosphoserine aminotransferase; q.d., one time per day; q.i.d., four times per day; RBC, red blood cell; s.c., subcutaneous injection; SEM, scanning electron micrograph; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SI, selectivity index; TD_{50}, median cytotoxic dose; TI, therapeutic index; TEM, transmission electron micrograph; t.i.d., three times per day; t.p., topical administration.

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ORIGINAL PAPER

Antibodies against *Clonorchis sinensis* LDH could cross-react with LDHB localizing on the plasma membrane of human hepatocarcinoma cell SMMC-7721 and induce apoptosis

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Abstract Lactate dehydrogenase (LDH) is a terminal enzyme in anaerobic glycolytic pathway. It widely exists in various organisms and is in charge of converting the glycolysis product pyruvic acid to lactic acid. Most parasites, including *Clonorchis sinensis*, predominantly depend on glycolysis to provide energy. Bioinformatic analysis predicts that the LDHs from many species have more than one transmembrane region, suggesting that it may be a membrane protein. *C. sinensis* LDH (CsLDH) has been confirmed as a transmembrane protein mainly located in the tegument. The antibodies against CsLDH can inhibit the worm's energy metabolism, kill the worm, and may have the same effects on human cancer cells. In this study, we cloned and characterized human LDHA (HsLDHA), HsLDHB, and CsLDH. Semi-quantitative real-time RCP showed that HsLDHB only existed in hepatocarcinoma cell SMMC-7721. Confocal microscopy and Western blot experiments revealed that HsLDHB was localized in the plasma membrane of SMMC-7721 cells, and the antibodies against CsLDH could cross-react with it. This cross-reaction could inhibit the enzymatic activity of HsLDHB. The cancer cells co-cultured with anti-CsLDH sera showed a significant decrease in cell proliferation rate and increases in caspase 9 and reactive oxygen species (ROS) levels. Therefore, anti-CsLDH antibodies can induce the apoptosis of cancer cells SMMC-7721 and may serve as a new tool to inhibit tumor.

Keywords Lactate dehydrogenase · Hepatocarcinoma · *Clonorchis sinensis* · Antibody cross-reaction

Introduction

Clonorchis sinensis, the oriental liver fluke, causes an important food-borne zoonosis in some Asian countries, including China, Japan, Korea, and Vietnam (Young et al. 2010). The

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REVIEW

Angiostrongylus cantonensis in the vector snails *Pomacea canaliculata* and *Achatina fulica* in China: a meta-analysis

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Abstract Angiostrongyliasis is a food-borne parasitic disease induced by the nematode *Angiostrongylus cantonensis*, and has been recognized as the main cause leading to human eosinophilic meningitis. Humans usually acquire infection by digestion of infected *Pomacea canaliculata* and *Achatina fulica*, the most predominant intermediate hosts found in China. This meta-analysis was aimed to assess the prevalence of *A. cantonensis* infection among these two snails in China in the past 10 years. Data were systematically collected in electronic databases such as PubMed, Web of Science, ScienceDirect, CNKI, SinoMed, VIP, CSCD, and Wanfang from 2005 to 2015. Thirty-eight studies with a total of 41,299 *P. canaliculata* and 21,138 *Ac. fulica* were included in the present study. The overall infection rate of *A. cantonensis* in China was estimated to be 7.6 % (95 % confidential interval (CI) = 0.063 to 0.090) in *P. canaliculata* and 21.5 % in *Ac. fulica* (95 % CI = 0.184 to 0.245), respectively. No significant difference was observed in prevalence rates among publication year and sample size for both snails. Also, it was found that the prevalence in *Ac. fulica* is significantly higher than that in *P. canaliculata* (odds ratio (OR) = 3.946, 95 % CI = 3.070 to 5.073). The present study reveals that snail infection with *A. cantonensis* is clearly prevalent in China. Further studies are required to improve strategies for control of infections of snails, particularly those of *Ac. fulica*, and to detect further factors and conditions such as geographic region, temperatures, and diagnosis method.

Keywords *Pomacea canaliculata* · *Achatina fulica* · *Angiostrongylus cantonensis* · Infection rates · Meta-analysis of occurrence

Langui Song, Xiaowen Wang and Zi Yang contributed equally to this work.

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Introduction

Angiostrongylus cantonensis, a food-borne zoonotic parasite, has been recognized as the primary pathogen associated with human eosinophilic meningitis or eosinophilic meningoencephalitis (Eamsobhana 2014). This neurotropic nematode has molluscan intermediate hosts such as raw apple snails (*Pomacea canaliculata*) and raw giant African land snails (*Achatina fulica*) (Chiu et al. 2014; Estebenet and Martín 2002) and uses as final hosts several species of rodents. The adult worms live in the pulmonary arteries of rats. Humans are non-permissive, accidental hosts, thus presenting severe central nervous system symptoms due to larvae migrans. Humans

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Parasites & Vectors

RESEARCH Open Access

Analysis of the mitochondrial maxicircle of *Trypanosoma lewisi*, a neglected human pathogen

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Abstract

Background: The haemoflagellate *Trypanosoma lewisi* is a kinetoplastid parasite which, as it has been recently reported to cause human disease, deserves increased attention. Characteristic features of all kinetoplastid flagellates are a uniquely structured mitochondrial DNA or kinetoplast, comprised of a network of catenated DNA circles, and RNA editing of mitochondrial transcripts. The aim of this study was to describe the kinetoplast DNA of *T. lewisi*.

Methods/Results: In this study, purified kinetoplast DNA from *T. lewisi* was sequenced using high-throughput sequencing in combination with sequencing of PCR amplicons. This allowed the assembly of the *T. lewisi* kinetoplast maxicircle DNA, which is a homologue of the mitochondrial genome in other eukaryotes. The assembly of 23,745 bp comprises the non-coding and coding regions. Comparative analysis of the maxicircle sequence of *T. lewisi* with *Trypanosoma cruzi, Trypanosoma rangeli*, *Trypanosoma brucei* and *Leishmania tarentolae* revealed that it shares 78 %, 77 %, 74 % and 66 % sequence identity with these parasites, respectively. The high GC content in at least 9 maxicircle genes of *T. lewisi* (*ATPase6*; NADH dehydrogenase subunits *ND3*, *ND7*, *ND8* and ND9; G-rich regions *GR3* and *GR4*; cytochrome oxidase subunit *COIII* and ribosomal protein *RPS12*) implies that their products may be extensively edited. A detailed analysis of the non-coding region revealed that it contains numerous repeat motifs and palindromes.

Conclusions: We have sequenced and comprehensively annotated the kinetoplast maxicircle of *T. lewisi*. Our analysis reveals that *T. lewisi* is closely related to *T. cruzi* and *T. brucei*, and may share similar RNA editing patterns with them rather than with *L. tarentolae*. These findings provide novel insight into the biological features of this emerging human pathogen.

Keywords: *Trypanosoma lewisi*, Kinetoplast maxicircle, Mitochondrial DNA, RNA editing, Palindrome

Background

The genus *Trypanosoma* belongs to the Kinetoplastea, which lies within the eukaryotic supergroup Excavata and comprises an assembly of mostly parasitic flagellated protists [1]. The best known trypanosomes are the human pathogenic *Trypanosoma brucei gambiense* and *T. b. rhodesiense* causing sleeping sickness in Africa, and *T. cruzi*, the causative agents of Chagas disease in South America. Other members of this genus are economically important animal parasites. *Trypanosoma lewisi* has long been recognized as a globally distributed obligatory parasite of rodents of the genus *Rattus*, transmitted by rat fleas and non-pathogenic to its natural hosts and humans [2]. This view has changed recently when human infections were reported [3] which culminated with a case of a fatal infection in an infant with a *T. lewisi*-like flagellate [4]. More importantly, the resistance of this parasite to the lysis by normal human serum was recently demonstrated [5]. These reports substantially raise the importance of this flea-transmitted trypanosome, which can now be considered a neglected human parasite [3, 6–8]. This is particularly important in developing countries where infants may have encountered direct

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PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Albendazole and Corticosteroids for the Treatment of Solitary Cysticercus Granuloma: A Network Meta-analysis

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Abstract

Background

Solitary cysticercus granuloma (SCG) is the commonest form of neurocysticercosis in the Indian subcontinent and in travelers. Several different treatment options exist for SCG. We conducted a Bayesian network meta-analysis of randomized clinical trials (RCTs) to identify the best treatment option to prevent seizure recurrence and promote lesion resolution for patients with SCG.

Methods and Principal Findings

PubMed, EMBASE and the Cochrane Library databases (up to June 1, 2015) were searched for RCTs that compared any anthelmintics or corticosteroids, alone or in combination, with placebo or head to head and reported on seizure recurrence and lesion resolution in patients with SCG. A total of 14 RCTs (1277 patients) were included in the quantitative analysis focusing on four different treatment options. A Bayesian network model computing odds ratios (OR) with 95% credible intervals (CrI) and probability of being best (P_{best}) was used to compare all interventions simultaneously. Albendazole and corticosteroids combination therapy was the only regimen that significantly decreased the risk of seizure recurrence compared with conservative treatment (OR 0.32, 95% CrI 0.10–0.93, P_{best} 73.3%). Albendazole and corticosteroids alone or in combination were all efficacious in hastening granuloma resolution, but the combined therapy remained the best option based on probability analysis (OR 3.05, 95% CrI 1.24–7.95, P_{best} 53.9%). The superiority of the

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LETTERS TO THE EDITOR

Aerosolized avian influenza A (H5N6) virus isolated from a live poultry market, China

Dear Editor,

Recent articles in this Journal have referred to avian influenza H5N6 infections in China[1—3] and examination of the Global Initiative on Sharing All Influenza Data (GISAID) database, one observes that more than 90 percent of China's recent laboratory-confirmed human infections with avian H7N9 or H5N6 influenza A viruses have been associated with exposure to live poultry or live poultry markets (LPMs). While cross-species poultry-to-person avian influenza transmission has been well documented[4,5] and avian influenza virus (AIV) can be transmitted between mammals, few studies have evaluated aerosolization of AIV within LPMs.[6] Numerous successful studies described to collect airborne microbes with bioaerosol sampling in different environments.[7] Influenza A (H1N1, H1N2 and H3N2 subtypes) virus infectious aerosols were detected in the air samples and that these aerosols could be exhausted from pig barns and be transported downwind.[8] To survive on the risk presence of airborne AIV in LPMs and provide potential clue in circulation of AIV, we conducted bioaerosol surveillance for AIV among LPMs located in Zhongshan, Guangdong, China.

During January 13, 2015 to April 20, 2016, weekly bioaerosol surveillance for AIV was conducted in 3 of Zhongshan's LPMs. The sampling technique was as previously described.[9] Air samples were collected using SKC Biosamplers (SKC Inc., Eighty Four, PA; catalog number 225-9595) attached to SKC BioLite Pumps (SKC Inc., Eighty Four, PA; catalog number 228-9610). The collection medium consisted of 15 ml of phosphate buffered saline (PBS) with 0.5% (w/v) bovine serum albumin fraction V powder (Sigma—Aldrich, St. Louis, MO), and an adjusted collection flow rate which is suitable for maintaining viability of captured viruses of 8 L/min for 30 min was used. Biosamplers were placed at a collection height of 0.8 m above the ground and 0.2 m away from the poultry cages which were randomly selected in the LPMs. Collected samples were hand-carried on ice to the Zhongshan Center for Disease Control and Prevention viral laboratory where they were concentrated using Amicon Ultra-15 Centrifugal Filter Units (EMD Millipore, Billerica, MA) at 2500 g for 15 min. Total nucleic acid was extracted using the QIAxtractor (Qiagen, Inc., Venlo, The Netherlands) and then tested for influenza A virus RNA (vRNA) using a real-time reverse transcription polymerase chain reaction (rRT-PCR).[9]

Positive samples were inoculated into embryonated chicken eggs, and the allantoic fluid harvested after incubation of the inoculated eggs for 2 days. The harvested allantoic fluid was then retested with rRT-PCR. Whole genome sequencing of the harvested virus was accomplished using a MiSeq (Illumina Inc., USA).

Genomic sequences (GenBank accession numbers, KX223685 through KX223692) were assembled and analyzed by CLC Genomics workbench (CLC Bio). Deduced amino acid sequences alignment and dendrogram analysis of the full-length genomic sequences with homologs were carried out by using the software of MEGA software (version 6). Phylogenetic trees were constructed using the maximum likelihood method with 1000 bootstrap tests.

Of the 243 air samples collected, 19 (7.8%) were initially positive for influenza A vRNA. One sample collected February 29, 2016 was positive by rRT-PCR post-inoculation in embryonated chicken eggs, and identified as an influenza A (H5N6) virus by whole-genome sequencing. This identified strain was designated A/Environment/Zhongshan/ZS01/2016(H5N6) (abbreviated as ZS01 in this manuscript).

Phylogenetic analysis of the hemagglutinin (HA) gene revealed that the virus isolate belonged to clade 2.3.4.4. (Fig. 1A). The HA protein of strain ZS01 has multiple basic amino acids at the HA cleavage site (PLRERRRKR/GLF), which is characteristic of highly pathogenic AIV. Furthermore, no mutations that encode Q222L or G224S (H5 numbering system) were found within the HA gene, suggesting that the virus had an avian-like (a2, 3-SA) receptor binding preference. However, several mammalian-adaptive mutations and species associated signature positions were found within HA (I151T, S123P, T156A) (Supplementary Table 1). The HA and NA genes of ZS01 exhibited high identities in coding sequence with A/chicken/Zhejiang/727155/2014 (H5N6) (98.83% and 97.03% respectively) and with A/chicken/Shenzhen/1061/2013(H5N6) (98.36%, 98.48% respectively) (Fig. 1A, B). HA and NA genes also demonstrated high identities with human H5N6 isolates (Supplementary Table 2). PB1, NP, PA, MP, NS genes of ZS01 were located in the H5 clade, Eurasian lineage (Supplementary Fig. 1A—F), consistent with China's human H5N6 isolates.[1,3]

The neuraminidase (NA) gene shared high identities with those of Guangdong poultry H6N6 strains (Supplementary Table 2). The PB2 gene of ZS01 shared 99.2% identity with

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Activation of GPER suppresses epithelial mesenchymal transition of triple negative breast cancer cells via NF-κB signals

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ABSTRACT

The targeted therapy for triple-negative breast cancer (TNBC) is a great challenge due to our poor understanding on its molecular etiology. In the present study, our clinical data showed that the expression of G-protein coupled estrogen receptor (GPER) is negatively associated with lymph node metastasis, high-grade tumor and fibronectin (FN) expression while positively associated with the favorable outcome in 135 TNBC patients. In our experimental studies, both the *in vitro* migration and invasion of TNBC cells were inhibited by GPER specific agonist G-1, through the suppression of the epithelial mesenchymal transition (EMT). The G-1 treatment also reduced the phosphorylation, nuclear localization, and transcriptional activities of NF-κB. While over expression of NF-κB attenuated the action of G-1 in suppressing EMT. Our data further illustrated that the phosphorylation of GSK-3β by PI3K/Akt and ERK1/2 mediated, at least partially, the inhibitory effect of G-1 on NF-κB activities. It was further confirmed in a study of MDA-MB-231 tumor xenografts in nude mice. The data showed that G-1 inhibited the *in vivo* growth and invasive potential

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Research Paper

A recombinant chimeric protein specifically induces mutant KRAS degradation and potently inhibits pancreatic tumor growth

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ABSTRACT

Pancreatic cancer is one of the most lethal human diseases, with an all-stage 5-year survival rate below 5%. To date, no effective and specific therapy is available for this disease. Mutations in KRAS are frequently reported in pancreatic and many other cancers; thus, KRAS is an attractive therapeutic target. Our objective was to specifically eliminate mutant KRAS and induce cell death of tumors expressing this mutant protein. We thus constructed several chimeric proteins by connecting the C-terminal domains of several adaptor proteins of E3 ubiquitin ligases such as CBL, CHIP, E6AP, and VHL, as well as VIF encoded by human immunodeficiency virus type 1 (HIV-1), to the Ras binding domain (RBD) of Raf. Although all of these chimeric proteins caused the degradation of mutant KRAS and the death of KRAS-mutant-tumor cell lines, the RBD-VIF with a protein transduction domain (PTD), named PTD-RBD-VIF, had the strongest tumor-killing effect. Intraperitoneally administered recombinant PTD-RBD-VIF potently inhibited the growth of xenografted KRAS-mutant pancreatic cancer cells. Our findings indicate that recombinant PTD-RBD-VIF, a chimeric protein with a combined cellular-viral origin, could be further developed for the treatment of various tumors harboring mutant or over-activated KRAS, especially for cases presenting with pancreatic cancer recurrence after surgery.

INTRODUCTION

Pancreatic carcinoma is an aggressive cancer and early diagnosis and radical surgery provide the only chance of long-term survival for patients [1–3]. A important feature of this cancer is that most pancreatic cancer cells harbor oncogenic mutations in the KRAS gene in the early stage, and the mutant KRAS is required to initiate pancreatic carcinoma [4, 5]. Substantial evidence has demonstrated the complexity of oncogenic KRAS signaling in promoting pancreatic cancer [6, 7]. Besides, many other tumors harbor mutations in the KRAS gene. In pancreatic cancer, colon cancer, and non-small cell lung cancer, the KRAS mutation rates are 90%, 45%, and 35%, respectively [8, 9]. Normal KRAS proteins function as molecular switches that cycle between the GDP-bound inactive and the GTP-bound active forms [10]. When bound to GTP, they interact with the downstream protein Raf and transduce the signal to activate various cell activities such as proliferation, differentiation, apoptosis, and migration [11, 12]. Specific point mutations in KRAS, especially those at position 12, maintain KRAS in its GTP-bound active form and consequently lead to tumor formation [13]. A variety of studies have been focused on the novel anti-kinase agents, which target the downstream kinases of KRAS signaling pathways such as MEK, PI3K and AKT [14–16]. Some of the inhibitors for these kinases are in clinical trials. However, the direct therapeutic agents for the inhibition of mutant KRAS is rare and urgently needed for the treatment of this disease.

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A comprehensive analysis of membrane and morphology of erythrocytes from patients with glucose-6-phosphate dehydrogenase deficiency

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ABSTRACT

Acute hemolytic anemia could be triggered by oxidative stress in the patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. However, the underlying hemolytic mechanism is unknown. To make clear the hemolytic mechanisms, a systematic study on membrane ultrastructure had been undertaken. A comprehensive method was used including atomic force microscopy, scanning electron microscopy, flow cytometer and fluorescence microscopy to analyze the membrane ultrastructure, externalized phosphatidylserine (PS), intracellular Ca^{2+} concentration, morphology and the distributions of band 3 protein in G6PD deficient red blood cells (RBCs) after tert-butyl-hydroperoxide (t-BHP) oxidation. The results showed that erythrocyte shrinkage, annexin-V binding to externalized PS on the membrane of early-stage apoptotic cells, the increased membrane roughness and intracellular Ca^{2+} concentration, as well as the change of distributions of band 3 protein in RBCs. Compared with the control RBCs, as the concentration of t-BHP up to 0.1 mM, the membrane roughness of G6PD deficient RBCs showed significant difference ($p < 0.05$) and as the concentration of t-BHP up to 0.3 mM, externalized PS showed significant difference ($p < 0.05$). Furthermore, the population types of RBCs showed dramatic difference between control groups and G6PD deficient groups. Oxidative stress induced more serious erythrocyte apoptosis and resulted in increased roughness of erythrocyte membrane and abnormal distributed band 3 protein in G6PD deficient RBCs. Echinocytes are the predominant abnormal erythrocyte shape occurring in the peripheral blood from patients with G6PD deficiency, which may shorten the RBCs lifespan. The results in the present study will give an increased understanding for the hemolytic mechanism of G6PD deficiency.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is an X-linked enzyme that catalyzes the first rate-limiting step in the pentose phosphate pathway, producing reduced nicotinamide adenine dinucleotide phosphate (NADPH), an obligatory substrate for several redox systems, in particular for glutathione (Beutler, 1996; Nkhoma et al., 2009). Red blood cells (RBCs) of G6PD deficient subjects are vulnerable to oxidative stress which predisposes them to chemical-induced hemolysis when some drugs like primaquine or other oxidative agents were administered (Ashley et al., 2014). G6PD deficiency is the most common human enzymopathy affecting more than 400 million people worldwide (Jiang et al., 2006). As a consequence, it is crucial importance to make clear the hemolytic mechanisms, ultimately to protect G6PD deficient subjects from oxidative damage (Manganelli et al., 2013).

During the past decade, accumulating evidence has demonstrated that G6PD deficiency affects cellular pathophysiological events in nucleated cell, such as accelerated cellular senescence (Au et al., 2002; Lee et al., 2011). Interesting, erythrocytes devoid of nuclei, mitochondria and other important organelles are capable of undergoing some of the morphological features of apoptosis, such as external exposure of phosphatidylserine (PS), membrane blebbing and cell shrinkage (Lang et al., 2003, 2012). The apoptosis

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; RBCs, red blood cells; AFM, atomic force microscopy; SEM, scanning electron microscopy; Ra, arithmetic average roughness; Rq, root-mean-square roughness; PS, phosphatidylserine; NADPH, nicotinamide adenine dinucleotide phosphate; t-BHP, tert-butyl-hydroperoxide.

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Infectious Diseases of Poverty

CASE STUDY Open Access

A case report: A rare case of infant gastrointestinal cantharisis caused by larvae of *Lasioderma serricorne* (*Fabricius,* 1792) (Coleoptera: Anobiidae)

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Abstract

Background: Cantharisis is a disease of humans caused by the infestation of beetle larvae. It is the second important insectal disease after myiasis. Several species of beetles are reported to cause the disease in gastrointestinal tract, urogenital system, nasal sinuses, ears and faces of mammals. The cigarette beetle *Lasioderma serricorne* is a widespread and destructive pest that usually feeds on tobacco, tea, beans, cereal grains, and animal and plant specimen. While there was no previous evidence of human infestation by this worm, we report the first case of *L. serricorne* infestation in a baby girl in China.

Case presentation: Here the case, an eight-month-old baby girl with irritable feeling, rubbing eyes, history of contact with mud and eating oranges twice during five days before attendance, and having "worms" in her stool was admitted to the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. The clinical examination revealed that the pulse rate, blood pressure and temperature were regular, and the examination of the head, neck, and chest were unremarkable. The stool specimens containing "worms" were sent to the Department of Parasitology, Zhongshan School of Medicine, Sun Yat-sen University. The worms were recovered, studied morphologically using naked eyes and anatomical lens, PCR analyzed targeting cytochrome oxidase subunit 1 (*COX1*) and 18S rRNA genes, examined by sequence analyses of the PCR products and finally classified by phylogenetic analysis to identify their species. Based on the findings, the worms were diagnosed as the larvae of *L. serricorne*.

Conclusion: This report implies that the baby had an infestation with the larvae of *L. serricorne* in the gastrointestine. During contact with mud or eating oranges by the girl, worm eggs were swallowed into the stomach and resisted gastric acid digestion which eventually hatched into larvae and caused cantharisis. The 8 months girl had underdeveloped immune system which might facilitate the disease. This report implicates that *L. serricorne* can infest human accidentally and cause cantharisis that may lead to severe damage to infant and older patient upon involvement of important organs of the body. The patients once diagnosed having cantharisis should be treated in time.

Keywords: Cantharisis, *Lasioderma serricorne*, Larvae, *COX1*, 18S rRNA

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