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临床经验 CLINICAL PRACTICE

肝癌患者血液RASSF1A基因甲基化的检测及其临床意义

仇小强, 陈 罡, 余红平, 胡 浪

■背景资料
肝癌(HCC)是我国发病率较高的恶性肿瘤之一, 在广西尤为高发, 目前仍无特异性的早期诊断指标. 本文通过探讨研究RASSF1A在HCC患者血清中甲基化的情况, 为研究HCC发生机制及寻求新的早期无创性肿瘤标志物提供一定的科学依据.

仇小强, 余红平, 胡浪, 广西医科大学公共卫生学院流行病学教研室 广西壮族自治区南宁市 530021
陈罡, 广西医科大学基础医学院病理教研室 第一附属医院病理科 广西壮族自治区南宁市 530021
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作者贡献分布: 此课题由仇小强、胡浪及余红平设计; 研究过程实验操作由仇小强、胡浪及陈罡完成, 仇小强负责技术指导; 数据分析由胡浪完成; 本论文写作由胡浪及陈罡完成, 仇小强及余红平负责论文修改工作.
通讯作者: 胡浪, 530021, 广西壮族自治区南宁市双拥路22号, 广西医科大学公共卫生学院流行病与卫生统计学教研室. hulang2000060@sina.com
电话: 0771-5358490
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Detection of RASSF1A promoter hypermethylation in plasma of patients with primary hepatocellular carcinoma and its clinical significance

Xiao-Qiang Qiu, Gang Chen, Hong-Ping Yu, Lang Hu

Xiao-Qiang Qiu, Hong-Ping Yu, Lang Hu, Department of Epidemiology and Statistics, Guangxi Medical University, Nanning 530021 Guangxi Zhuang Autonomous Region, China
Gang Chen, Department of Pathology, Guangxi Medical University, Nanning 530021 Guangxi Zhuang Autonomous Region, China
Supported by: the Medical Major Scientific Research Foundation of Guangxi Zhuang Autonomous Region, No. 200513
Correspondence to: Lang Hu, Department of Epidemiology and Statistics, Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China. hulang2000060@sina.com
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Abstract

AIM: To investigate the promoter methylation of Ras association domain family 1A (RASSF1A) in the serum of HCC and to explore the significance and value of the promoter methylation of RASSF1A as a new tumor molecular marker in early stage noninvasive diagnosis of HCC.

METHODS: Promoter methylation of RASSF1A status in the serum of HCC patients (n = 35) and normal controls (n = 10) were detected by methylation-specific PCR (MSP).

RESULTS: RASSF1A promoter methylation was detected in 14 cases (40%) in the serum from 35 HCC patients, while no RASSF1A methylation was detected in 10 normal controls. No association was found between serum RASSF1A methylation and the clinicopathological parameters, such as sex, para-cirrhosis, HBV, AFP, tumor size, tumor capsular, portal vein tumor embolus or pathological grade.

CONCLUSION: The promoter methylation of RASSF1A may play an important role in tumor genesis of HCC and act as a new tumor molecular marker for HCC.

Key Words: Hepatocellular carcinoma; Ras association domain family 1A; Promoter methylation; Serum

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摘要

目的: 探讨原发性肝细胞癌(HCC)患者血清中RASSF1A甲基化状况及RASSF1A甲基化作为一种新的肿瘤分子标志物在HCC早期无创性诊断中的意义和价值.

方法: 应用甲基化特异性PCR(MSP)技术检测35例HCC患者血清和10例健康对照血清中RASSF1A启动子区甲基化状况.

结果: 35例HCC患者血清中RASSF1A启动子区甲基化阳性率为40%, 10份健康对照血清中未出现RASSF1A基因甲基化. RASSF1A基因甲基化与HCC患者性别、伴肝硬化、乙肝表面抗原、甲胎蛋白、肿瘤大小、有无包膜、有无门静脉癌栓及病理分级等临床病理参数无关.

结论: RASSF1A基因甲基化在HCC的发生中起重要作用, RASSF1A基因甲基化可能是HCC新的肿瘤分子标志物.

■同行评议者
李瑗, 主任医师, 广西肿瘤研究所病理研究室; 杜祥, 教授, 复旦大学附属肿瘤医院病理科

关键词: 原发性肝细胞癌; RASSF1A; 启动子区甲基化; 血清

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0 引言

RAS相关区域家族1A(ras association domain family 1A gene, RASSF1A)是新近从3号染色体短臂克隆出来的新型候选抑癌基因, 因其表达产物中含有与Ras蛋白结构相关区域, 故而得名[1]. 该基因启动区异常甲基化而失活的现象在多种恶性肿瘤血液及组织标本中得到证实, 如肺癌[2]、胆囊癌[3]、乳腺癌[4-5]、肾癌[6]、前列腺癌[7-8]、子宫内膜癌[9]、膀胱癌[10]、胆管癌[11]、胃癌[12-13]、肠癌[13-14]等. RASSF1A的高甲基化为恶性肿瘤的诊断提供了新的角度. 原发性肝细胞癌(hepatocelluar carcinoma, HCC)是我国高发肿瘤之一, 但HCC早期诊断仍是当前研究的一个难点. 赵强子 *et al*[15], Yeo *et al*[16]及Zhang *et al*[17]在HCC患者血清中检测到了RASSF1A基因的高甲基化. 为了进一步证实HCC血清中RASSF1A基因启动子区CpG岛异常高甲基化对早期诊断的意义, 我们应用MSP技术检测35例HCC患者血清样本RASSF1A启动子甲基化状况, 探讨RASSF1A甲基化与HCC发生发展的关系.

1 材料和方法

1.1 材料 HCC血清标本35例为广西医科大学第一附属医院肝胆外科2006-02/2006-07患者治疗前标本, 患者均为病理学检查证实, 符合2001年全国肝癌学术会议制定的肝癌诊断标准[18]. 35例HCC患者中男29例(82.9%), 女6例(29.1%); 年龄为26-68(平均为50±14.7)岁; 乙型肝炎感染30例(85.7%). 病理证实HCC合并肝硬化23例(65.7%), 合并慢性肝炎21例(60%). 10例健康对照血清来自健康献血者, 其中男7例, 女3例, 平均年龄为48±13.2岁. 核酸提取试剂盒(DNA Blood Midi Kit)购自德国Qiagen公司; 基因修饰试剂盒(CpGenome™ DNA Modification Kit S7820)购自美国Chmicon公司; RASSF1A基因PCR扩增试剂盒(CpG WIZ RASSF1A Amplification Kit S7813)购自美国Chmicon公司.

1.2 方法

1.2.1 DNA 提取: 血清DNA提取按照DNA Blood Midi Kit说明书进行, 每份样本采用0.8 mL血清. 提取的DNA纯度用紫外分光光度计检测, 吸光度A_{260}/A_{280}比值在1.6-1.8之间, DNA样本立即进行实验或保存于-20℃.

1.2.2 基因修饰: 按CpGenome™ S7820试剂盒说明把胞嘧啶转化为尿嘧啶, 进行基因修饰.

1.2.3 甲基化特异性PCR(MSP)检测: 非甲基化特异引物(U)和甲基化特异引物(M)为针对RASSF1A基因启动子及第一外显子CpG岛序列设计, 由Chmicon公司提供. 其中U引物PCR产物为108 bp, M引物为111 bp. PCR扩增体系如下: 10×PCR Buffer 2.5 μL; 2.5 mmol/L dNTP 2.5 μL; U, M或W primers 1.0 μL; "hot start" enzyme(5 U/μL)0.2 μL; dH_2O 16.8 μL; Template DNA(50 mg/L)2.0 μL. 总体积25.0 μL. 反应条件: 95℃预变性5 min; 95℃变性(45 s)、55℃退火(45 s)、72℃延伸(60 s)共35 cycles; 最后72℃延伸5 min. PCR扩增产物10 μL进行20 g/L琼脂糖凝胶电泳, 若仅U引物能扩增出条带, 且目的条带位置正确(108 bp), 可判断为甲基化阴性; 若仅M引物出现条带(111 bp), 判断为完全甲基化; 如U和M引物出现条带, 且目的条带位置正确, 判为部分甲基化. 完全甲基化及部分甲基化均为甲基化阳性.

统计学处理 应用SPSS13.0统计软件对数据进行χ^2检验, 以$P<0.05$判为差异有显著性意义.

2 结果

HCC患者血清RASSF1A启动子区甲基化阳性率为40%(14/35), 10份健康对照血清中未见RASSF1A基因甲基化阳性. 血清RASSF1A基因甲基化发生情况与患者各临床病理参数无明显相关(表1).

3 讨论

2000年, Dammann *et al*[1]分离出一种能与DNA修补蛋白XPA相互作用的一种候选cDNA, 其核苷酸序列的碳端与鼠Ras效应蛋白Nore1和Maxp1高度同源, 遂命名为Ras相关区域家族1, 即RASSF1基因; 同时还发现, 由于该基因不同的剪接和使用启动子的不同, 存在3种不同的转录本(转录本A、B和C). 其中RASSF1A含1α和2αβ, 其cDNA全长1873 bp. 该基因出现异常, 如发生3p21区的杂合性缺失, 启动子区域CpG岛异常高甲基化而导致其转录"沉默"时, 影响其功能的发挥, 则可能导致肿瘤的形成[3,19-23]. RASSF1A基因在恶性肿瘤中频繁的甲基化的特点, 使之被广泛应用于肿瘤表遗传学研究[4,24-29].

■创新盘点
本文阐释了广西地区HCC患者血清中RASSF1A的甲基化情况, 为HCC的具体发生机制及早期诊断提供一定的科学依据.

■研发前沿
目前关于肿瘤的早期诊断的发展备受关注, 也是HCC的研究热点, HCC的早期诊断方法有待于进一步研究.

■相关报道
相关研究发现RASSF1A在多种恶性肿瘤的组织及血清中出现甲基化, 明显高于非癌组织及正常血清, RASSF1A有潜力成为新的肿瘤标记基因.

表 1 血清RASSF1A基因甲基化与HCC临床病理参数的关系

临床病理资料	*n*	阳性	χ^2值	*P*值
年龄				
≥50	16	6	0.077	0.782
<50	19	8		
性别				
男	28	12	0.476	0.490
女	7	2		
病理分级				
Ⅰ–Ⅱ	20	8	0.000	1.000
Ⅲ–Ⅳ	15	6		
HBV				
阳性	30	11	0.972	0.324
阴性	5	3		
伴肝硬化				
有	23	10	0.338	0.561
无	12	4		
AFP(μg/L)				
≥400	24	9	0.199	0.656
<400	11	5		
门脉癌栓				
有	6	2	0.134	0.714
无	29	12		
包膜				
有	14	5	0.179	0.673
无	21	9		
肿瘤直径(cm)				
≥5	26	11	0.224	0.636
<5	9	3		

肿瘤的早期无创诊断主要针对体液检查. 研究结果显示: 肿瘤患者体液中存在RASSF1A基因启动子区CpG岛异常甲基化, 为肿瘤患者的早期无创性诊断提供了新的思路. 研究发现: 50%肺癌患者痰液[30]、84%肺癌患者血清[31]、35%膀胱癌患者尿液[32]、56%-60%的乳腺癌患者血清[5,33-34]及5%的未分化鼻咽癌[35]中均可检测到RASSF1A异常甲基化. 因此检测体液中RASSF1A异常甲基化对肿瘤的无创性诊断有重要意义. 本研究对35例HCC患者血清进行RASSF1A基因启动子区CpG岛异常甲基化检测, 结果14例为阳性, 占总体40%, 10份健康对照血清无一例出现RASSF1A基因甲基化, 提示相对于正常对照, HCC患者血清中异常甲基化的游离RASSF1A基因与HCC发生有关. 本研究结果与赵强子 *et al*[15](41.5%, 17/41)及Yeo *et al*[16](42.5%, 17/40)的研究结果相近, 但却低于Zhang *et al*[17]的研究结果(70%, 35/50), 可能与病例数与病例来源有关. Yeo *et al*[16]发现HCC血清中RASSF1A基因甲基化与肿瘤大小有关, 肿瘤越大越容易发生RASSF1A甲基化, 但与其余临床病理参数无关. 本研究中血清RASSF1A基因甲基化与临床病理资料未显示相关性, 与赵强子 *et al*[15]结果一致, 提示HCC患者血清中RASSF1A基因甲基化可能是肿瘤形成的早期事件, 血清RASSF1A的检测有早期预示肿瘤的作用. 另外, 由于RASSF1A基因甲基化检测的肿瘤特异性及敏感性, 对术后患者进行血清RASSF1A基因甲基化检测有望提示肿瘤复发, 有助于HCC预后判断及及时治疗.

■应用要点
实验结果说明RASSF1A甲基化在HCC的发生中发挥作用, 检测RASSF1A甲基化指标可能有助于HCC早期诊断, 并为HCC临床基因治疗提供新的治疗思路.

总之, 本研究通过检测HCC血清中RASSF1A启动子区异常甲基化, 分析了RASSF1A甲基化与HCC发生的可能关系. RASSF1A基因甲基化可能成为HCC新的敏感的肿瘤分子标志物, 检测RASSF1A基因甲基化对HCC早期无创性诊断及判断复发有一定的临床意义和价值.

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编辑 李军亮 电编 吴鹏朕