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胃黏膜异型增生组织中微卫星不稳定性的检测及其意义的探讨

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Microsatellite instability in dysplasia mucosa and gastric cancer

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Abstract

AIM: Gastric mucosa dysplasia has been regarded as a precancerous lesion of the stomach. Abnormal gene alterations in the mucosa dysplasia of the stomach, which may lead to cell transformation, are probably of great importance in the development of gastric cancer (GC). Microsatellite instability (MSI) is a good marker of genome instability. Investigation of MSI in the cancer and precancerous lesion of the stomach will help us to search into the carcinogenesis of stomach and the potential role of MSI in the development of gastric cancer.

METHODS: Silver staining single strand conformation polymorphis-polymerease chain reaction (PCR-SSCP) was used to screen MSI markers at 5 loci in formalin-fixed, paraffin-embeded tissues of GC (n =30), dysplasia (n =30) and corresponding normal gastric tissues.

RESULTS: The abnormal shifting of the single-strand DNA was identified in 7 (23.3%) out of GC, in 9 (30%) out of dysplasia samples respectively. Three (10%) tumors and two (6.7%) dysplasia displayed a high-level of MSI (two or more loci altered). Low-level MSI (one loci altered) was detected in 13.3% of the tumors and in 23.3% dysplasia samples. GC with MSI was associated with distal location of the tumors (P =0.044). No association was detected between MSI and the grade of dysplasia.

CONCLUSION: The accumulation of MSI in the dysplasia of gastric mucosa may be an early molecular event in the development of gastric cancer. It contributes probably to the multistep gastric carcinogenesis.

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摘要

目的: 胃黏膜异型增生是胃癌的癌前病变, 胃癌的发生可能是胃黏膜异型增生过程中的一系列基因变异累积引起胃黏膜细胞发生恶性转化所致. 微卫星不稳定性(MSI)是DNA基因组不稳定的重要标志, 研究胃癌和癌前病变组织MSI的存在情况, 有助于从基因组不稳定的角度探讨胃癌可能的发生机制以及MSI在胃癌发生过程中的可能作用.

方法: 胃癌 30 例、异型增生组织 30 例分别提取病变组织及相应正常组织的 DNA, 应用银染 PCR-SSCP 技术检测5个微卫星位点不稳定性的存在状况.

结果: 胃癌组织 MSI 的发生率 23.3%, 胃窦癌 MSI 的发生率显著高于贲门癌(10% *vs* 3.3%, P = 0.044). 胃黏膜异型增生组织的 MSI 发生率 30%, MSI 与异型增生的程度无明显关系.

结论: MSI 是胃癌多步骤发生过程中的早期分子事件, 对胃癌的发生和发展可能具有重要作用.

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0 引言

胃癌的发生发展是一个多因素参与, 多基因变异累积, 逐渐演变的多阶段过程[1-16], 胃黏膜异型增生被认为是胃癌的癌前病变, 与胃癌的发生关系密切[17-23]. 微卫星不稳定(microsatellite instability, MSI)在多种肿瘤组织中均可检测到, 以胃癌发生率较高, 对于慢性胃炎基础上异型增生黏膜 MSI 的情况, 国内外报道均很少. 我们采用 PCR-SSCP 技术, 检测胃黏膜异型增生和胃癌两种组织中 MSI 的发生情况和特点, 探讨了 MSI 在胃癌发生发展中的可能作用.

1 材料和方法

1.1 材料 我院2001-01/2001-10因胃癌行手术切除者30例，均无胃癌家族史. 胃黏膜异形增生30例来自我院内镜室胃镜活检标本. 胃癌按1987年全国肿瘤会议PTNM分期标准分期，异型增生按WHO标准分轻、中、重度. 挑选分别包括病变及正常组织标本的蜡块，连续以5 μm厚度切片，用于DNA提取. 所有标本的病变均经病理医师确认. PCR引物、脱氧核苷酸(dNTPs)购自上海生工公司; Taq酶购自大连宝生物公司.

1.2 方法 石蜡切片经二甲苯脱蜡; 无水乙醇洗脱后烘干. 加入TE(Tris-EDTA，pH=8.0) 95 μL及蛋白酶K(终浓度500 mg/L) 5 μL，37 ℃过夜，离心后97 ℃ 7 min灭活蛋白酶K，取上清 – 20 ℃贮存. 引物序列参照相关文献的报道选用(Cancer Res 1997;57:4749 & 1998;58:5248，表1). PCR反应总体积25 μL，含50-200 ng DNA. 反应条件: 95 ℃ 5 min，94 ℃ 30 s，55-58 ℃ 30 s，72 ℃ 15 s，35个循环，72 ℃延伸1 min. 取PCR产物12 μL加变性缓冲液(980 mL/L去离子甲酰胺，20 mmol/L EDTA，0.1 g/L溴酚蓝，0.1 g/L二甲苯青)12 μL，97 ℃变性7 min，迅速置于乙醇碎冰中，双链对照标本用PCR产物6 μL加上样缓冲液(2.5 g/L溴酚蓝、400 g/L蔗糖)2 μL. 采用70 g/L非变性聚丙烯酰胺凝胶(19∶1)电泳，1 × TBE缓冲液，室温下180V恒压电泳3 h，银染制成干胶. MSI判断标准: 肿瘤组织与正常组织相比，出现异常条带或条带迁移. 阳性标本重复1次试验以确认无误.

统计学处理 各组之间年龄的比较采用单因素方差分析及*t*检验; 各组MSI发生率的比较采用Fisher精密检验，以P <0.05为差异有统计学显著性.

表1 引物序列

微卫星位点	引物序列	扩增片段大小
BAT-25	5’-TCGCCTCCAAGAATGTAAGT-3’	~90 bp
	5’-TCTGCATTTTAACTATGGCTC-3’	
BAT-26	5’-TGACTACTTTTGACTTCAGCC-3’	80-100 bp
	5’-AACCATTCAACATTTTTAACCC-3’	
D5S346	5’-ACTCACTCTAGTGATAAATCG-3’	96-122 bp
	5’-AGCAGATAAGACAGTATTACTAGTT-3’	
D17S250	5’-GGAAGAATCAAATAGACAA-3’	~150 bp
	5’-GCTGGCCATATATATATTTAAACC-3’	
D2S123	5’-AAACAGGATGCCTGCCTTTA-3’	197-227 bp
	5’-GGACTTTCCACCTATGGGAC-3’	

2 结果

在30例胃癌中有7例表现1个或1个以上位点的不稳定性(图1)，MSI发生率为23.3%，其中3例表现2个位点(大于或等于30-40%位点数)的不稳定性，记作MSI-H; 4例仅在一个位点表现不稳定性，记作MSI-L; 其余23例为MSS.

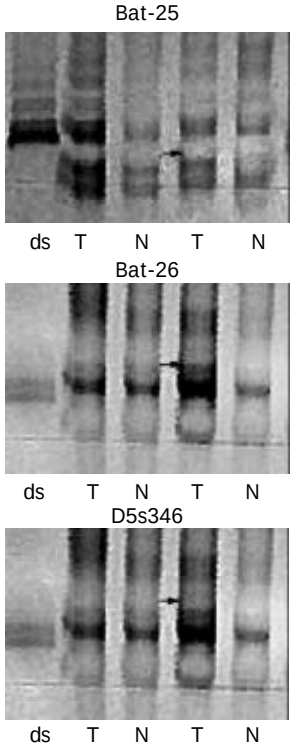

图1 胃癌组织MSI检出情况. Ds: 双链DNA; T: 胃癌组织; N: 正常组织; T与N相比, 多出一异常条带, 表明MSI的存在(箭头所指处).

2.1 MSI与临床病理的关系 MSI胃癌与MSS胃癌在性别、年龄、细胞分化程度、淋巴结转移及TNM分期方面无统计学差异，但胃窦癌较贲门癌更易表现MSI (P = 0.044，表2).

表2 胃癌组织MSI与临床病理的关系

特点	MSS (n = 23)	MSI-L (n = 4)	MSI-H (n = 3)
平均年龄(mean±SD)	57 ± 11	58 ± 5	69 ± 5
男 ： 女	17∶6	4∶0	2∶1
高中分化	5	1	2
低分化	18	3	1
贲门	12	1	0
胃体	9	2	1
胃窦[a]	2	1	2
淋巴结 –	10	2	3
+	13	2	0
TNM分期 Ⅰ，Ⅱ期	11	2	2
Ⅲ，Ⅳ期	12	2	1

[a] P <0.05 (Fisher精密检验), *vs* 贲门癌.

2.2 异型增生组织MSI 在30例慢性胃炎伴异型增生患者中9例存在MSI(图2)，阳性率为30%，其中7例为MSI-L，2例为MSI-H. 中度以上异型增生组织中MSI阳性率33.3%(6/18)，高于轻度异型增生的25%(3/12)，但二者之间的差异无统计学意义. MSI与MSS的异型增生在性别、年龄方面亦无显著性差异.

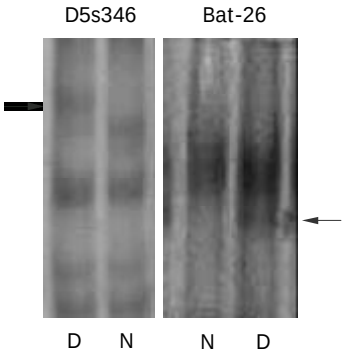

图2 异型增生组织的MSI及检出情况.D: 异型增生组织; N: 正常组织; D与N相比, 条带迁移率明显改变或出现一异常条带,表明MSI的存在(箭头所指处).

3 讨论

微卫星(microsatellite, MS)序列的多态性由等位基因间重复单位数目变化所引起. MSI是指由于复制错误引起的简单重复序列的改变, 表现为病变组织与其相应的正常组织相比, DNA结构性等位基因的大小发生改变. 其发生原因可能与DNA错配修复基因的缺陷有关. 在人类散发性肿瘤中, 胃癌MSI的发生率最高, 通常报道在13-44%, 个别达到76.7%[24-25]. 由于不同研究所选择的MS位点种类及数量不同, 所报道结果的可比性不强. 随着检测位点数量的增加, MSI阳性的机会也就增加. 我们选用1997年美国癌症研究院推荐的用于胃癌MSI检测的5个位点, 即2个单核苷酸, 3个二核苷酸重复序列, 胃癌MSI的发生率为23.3%. Leung *et al*[24]用以上5个位点加BAT-40, D13S170, TP53等3个位点分析30例胃癌组织的MSI, 阳性率达76.7%. 胃癌MSI阳性率的高低不一可能与所选位点的种类和数目、样本数量、MSI阳性的判断标准、遗传背景以及地理区域等不同有关.

MSI胃癌与临床病理的关系目前尚未明确, 一些研究资料显示[26-30], MSI-H与MSI-L和MSS胃癌相比具有不同的临床病理特征, MSI-H胃癌一般多发生在胃窦部, 少有淋巴结转移, 恶性程度较低, 预后较好. 本组结果表明, MSI与性别、年龄、肿瘤浸润深度、细胞分化程度、淋巴结转移、Lauren分型以及预后无明显相关. 由于本组资料病例数较少, 所得到的结果尚需更多样本的研究加以证实. 胃黏膜异型增生被认为是胃癌的癌前病变. 部分胃癌的发生可能是胃黏膜异型增生过程中一系列基因变异的累积所致. 因此, 研究胃癌和癌前病变组织MSI的存在情况, 可以从基因组不稳定的角度探讨胃癌可能的发生机制. 本组异型增生组织中MSI发生率为30%, 且与异型增生的程度无关, 这与Lee *et al*[31]报道的结果相似. 本研究结果显示, MSI在胃癌和癌前病变组织中均有相当比例的存在, 提示在癌前病变阶段已出现基因的不稳定现象, 这是胃癌发生前的分子异常事件, 可能会成为胃癌多步骤发生过程中的起点. Kashiwagi *et al*[32]对6例MSI的高分化型癌或胃腺瘤标本作回顾性研究, 取其1-7 a前的标本, 结果发现该6例标本在慢性胃炎阶段就已经表现为MSI, 提示MSI的出现在胃癌发生过程中发挥了重要作用, 是胃癌发生因素中的重要环节, 胃黏膜组织MSI的分析可能将有助于胃癌发生危险性的预测.

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• 消息 •

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《*World Journal of Gastroenterology*，WJG》编辑委员会由来自53个国家的179位消化病学专家组成，具有广泛的代表性，特别是聘请了一批工作在临床和科研一线，具有良好科学记录的中青年专家为评委. 分别来自以下国家: ALBANIA(1)，ALGERIA(1)，ARGENTINA(2)，AUSTRIA(1)，BELARUS(1)，BELGIUM(1)，BRAZIL(1)，CANADA(1)，CHINA(38)，COSTA RICA(1)，DENMARK(1)，EGYPT(1)，FINLAND(1)，GERMANY(7)，GREECE(1)，HUNGARY(2)，ICELAND(1)，INDIA(2)，IRAN(1)，IRELAND(1)，ISRAEL(1)，ITALY(2)，JAPAN(3)，KENYA(1)，LATVIA(1)，LITHUANIA(1)，MACEDONIA(1)，MALAYSIA(2)，MONACO(1)，NEW ZEALAND(1)，PHILIPPINES(1)，POLAND(2)，ROMANIA(1)，RUSSIA(3)，SINGAPORE(1)，SLOVENIA(3)，SOUTH AFRICA(1)，SOUTH KOREA(2)，SPAIN(1)，SRI LANKA(1)，SWEDEN(2)，SWITZERLAND(2)，THAILAND(1)，THE NETHERLANDS(1)，TURKEY(1)，UNITED KINGDOM(7)，UNITED STATES(55)，YUGOSLAVIA(1).WJG编委的任务是针对论文的科学性、创新性和先进性及可读性进行评价和修改，并针对我国现阶段科学研究整体水平和科研人员英文报告撰写能力的实际情况对稿件提出具体的建设性意见; 对投送WJG稿件的题名、摘要、引言、材料和方法、结果和讨论等进行综合评价，根据是否能较好地反映我国或国际消化病学临床和基础的先进水平决定所投论文是否被接受. 目前，投送WJG稿件的质量差别较大，有些论文的科学性、可读性方面的问题较多. 为此，WJG制定了稿件评审要点，对控制WJG的学术质量起到了核心作用.