PO Box 2345 Beijing 100023, China
Fax: +86-10-85381893
Email: wcjd@wjgnet.com www.wjgnet.com

World Chin J Digestol 2004 August;12(8):1774-1777
世界华人消化杂志 ISSN 1009-3079 CN 14-1260/R

胃癌微卫星不稳定与错配修复蛋白表达的缺失

李建华, 石先哲, 吕 申, 刘 敏, 王朝晖, 刘丽娜, 姜 静, 许国旺

李建华, 吕申, 刘敏, 王朝晖, 大连医科大学附属第二医院实验中心 辽宁省大连市 116023
石先哲, 许国旺, 中国科学院大连化学物理研究所, 国家色谱研究分析中心 辽宁省大连市 116011
刘丽娜, 大连医科大学附属第一医院消化内科 辽宁省大连市 116001
姜静, 大连理工大学材料系 辽宁省大连市 116023
李建华, 女, 1967-01-09生, 辽宁省彰武县人, 满族, 1990年中国医科大学本科毕业, 1993年大连医科大学硕士研究生毕业, 现在大连医科大学攻读博士, 主要从事分子病理学研究.
中国科学院知识创新工程领域前沿资助项目, No. DICPK2001A4
项目负责人: 吕申, 116023, 辽宁省大连市中山路465号, 大连医科大学附属二院实验中心分子生物学实验室. ljhsxw@pa18.com
电话: 0411-84687554
收稿日期: 2004-04-15 接受日期: 2004-05-09

Microsatellite instability and loss of mismatch-repair protein expression in gastric carcinoma

Jian-Hua Li, Xian-Zhe Shi, Shen Lv, Min Liu, Zhao-Hui Wang, Li-Na Liu, Jing Jiang, Guo-Wang Xu

Jian-Hua Li, Shen Lv, Min Liu, Zhao-Hui Wang, Laboratory of Molecular Biology, the Second Hospital of Dalian Medical University, Dalian 116023, Liaoning Province, China
Xian-Zhe Shi, Guo-Wang Xu, National Chromatographic Research and Application Center, Dalian Institute of Chemical Physics, the Chinese Academy of Sciences, Dalian 116011, Liaoning Province, China
Li-Na Liu, Department of Gastrointestine, the First Hospital of Dalian Medical University, Dalian 116001, Liaoning Province, China
Jing Jiang, Department of Material Engineering, Dalian Science and Technology University, Dalian 116023, Liaoning Province, China
Supported by the Knowledge Innovation Program of the Chinese Academy of Sciences, No. DICP K2001A4
Correspondence to: Shen Lv, Labortory Center of Molecular Biology. The Second Hospital of Dalian Medical University. Dalian 116023, Liaoning Province, China. ljhsxw@pa18.com
Received: 2004-04-15 Accepted: 2004-05-09

Abstract

AIM: To detect the microsatellite instability (MSI) and expression of mismatch repair (MMR) gene in gastric cancinoma, and to explore the molecular biological mechanism underlying the carcinogenesis of gastric cancinoma.

METHODS: A total of 56 cases of gastric cancinomas and surrounding non-cancerous tissues from surgical excision samples were collected, among which 22 cases were well and 34 cases were poorly differentiated adenocarcinoma, 20 cases were in early stage and 36 cases were in advanced stage of the disease. The microsatellite locus of BAT-26, D17S261, D3S1283, D2S123 and D3S1611 were amplified by PCR after DNA abstraction. Then PCR products were mixed together with GeneScan 500 size standard followed by heat denaturation. Microsatellites were analyzed by capillary electrophoresis with 6% SLPA and 8 moL/L^{-1} urea as sieving medium. Carcinoma were characterized as high MSI (MSI-H) if they manifested instability at two or more markers, low MSI (MSI-L) if unstable at only one marker, and microsatellite stable (MSS) if they showed no instability at any marker. Expression of MMR gene hMLH1 and hMSH2 were detected by immunohistochimical staining using the streptavidin-biotin-peroxidase complex method with 3, 3'-diaminobenzidine as chromogen.

RESULTS: Of the 56 cases of gastric carcinomas, 14 cases (25%) showed MSI-H and 14 cases (25%) showed loss of MMR. In the 14 cases of the MSI, 11 cases (79%) were accompanied by loss of hMLH1/hMSH2 expression, whereas In the 42 cases of the MSI-L/MSS, only 3 cases (7%) were accompanied by loss of hMLH1/hMSH2 expression. MSI was significantly related with mismatch repair deficiency (P <0.01). Of the 22 cases of well-differentiated carcinomas, 7 cases (32%) manifested MSI-H and 6 (27%) cases showed protein defection of MMR, Comparatively, 7 cases (21%) manifested MSI-H and 8 cases (24%) showed protein defection of MMR in 34 cases poorly-differentiated carcinomas. Of the 20 cases early stage carcinomas, only 1 cases (5%) manifested MSI-H and 3 (15%) cases showed protein defection of MMR, whereas 13 cases (36%) manifested MSI-H and 11 cases (31%) showed protein defection of MMR in 36 cases advanced carcinomas. The MSI frequency was higher in advanced stage (36%) than that in early stage (5%) of gastric carcinoma and the difference was significant (P <0.05), but no difference between well and poorly differentiated gastric carcinoma. The difference of loss frequency of hMLH1/hMSH2 expression was not significant in different stage and different differentiation of gastric carcinoma.

CONCLUSION: The defect of mismatch repair may be involved in the carcinogenesis of a subset of gastric cancer but not in the biologic behavior. MSI frequency increases with the progression of gastric carcinoma.

Li JH, Shi XZ, Lv S, Liu M, Wang ZH, Liu LN, Jiang J, Xu GW. Microsatellite instability and loss of mismatch-repair protein expression in gastric carcinoma. Shijie Huaren Xiaohua Zazhi 2004;12(8):1774-1777

摘要

目的: 分析胃癌组织微卫星不稳定性、错配修复蛋白表达以及二者的关系,探讨胃癌发生的分子生物学机制.

方法: 胃腺癌及癌旁组织标本56例, 高分化癌22例, 中低分化癌34例, 早期癌20例, 中晚期癌36例, 常规酚-氯仿法提取DNA, 选取基因组上的五个微卫星位点BAT-26、D17S261、D3S1283、D2S123和D3S1611, 进行PCR扩增, 扩增产物加入GeneScan 500 size standard共同热变性后, 用60 g/L的SLPA添加8mol/L尿素做筛分递质的毛细管电泳进行分析. 被检测的五个微卫星位点如出现两个或两个以上位点的不稳定, 定为微卫星的高

度不稳定(MSI-H), 一个位点出现不稳定、定为微卫星的低度不稳定(MSI-L), 没有位点出现不稳定、定为微卫星稳定(MSS). 石蜡切片常规免疫组织化学SP方法检测hMLH1和hMSH2蛋白的表达, 肿瘤组织上皮细胞核不着色, 而周围组织上皮细胞核着色判定为hMLH1或hMSH2蛋白表达的缺失.

结果: 在56例胃腺癌中, 有14例(25%)表现为MSI-H, 14例(25%)有错配修复蛋白表达的缺失. 在14例MSI-H的胃癌组织中, 11例(79%)有hMLH1或hMSH2表达的缺失, 42例MSI-L/MSS的胃癌组织仅3例(7%)有hMLH1或hMSH2表达的缺失. 胃癌的MSI-H与错配修复蛋白表达的缺失高度相关($P<0.01$). 其中的22例高分化腺癌有7例(32%)表现为MSI-H、6例(27%)有错配修复蛋白表达的缺失, 34例中低分化腺癌7例(21%)表现为MSI-H、8例(24%)有错配修复蛋白表达的缺失, 20例早期腺癌有1例(5%)表现为MSI-H、3例(15%)有错配修复蛋白表达的缺失, 36例中晚期腺癌13例(36%)表现为MSI-H、11例(31%)有错配修复蛋白表达的缺失. 微卫星不稳定性在中晚期胃癌明显高于早期胃癌($P<0.05$), 但在不同分化程度的胃癌差异不明显; MMR蛋白表达缺失在高分化与低分化以及早期与中晚期的胃癌均无显著性差异.

结论: 细胞错配修复功能缺陷与部分胃癌的发生有关, 而与胃癌的生物学行为无关; 胃癌的微卫星不稳定性随着肿瘤的演进而增加.

李建华, 石先哲, 吕申, 刘敏, 王朝晖, 刘丽娜, 姜静, 许国旺. 胃癌微卫星不稳定与错配修复蛋白表达的缺失. 世界华人消化杂志 2004;12(8):1774-1777
http://www.wjgnet.com/1009-3079/12/1774.asp

0 引言

胃癌是一种发病率和死亡率都很高的一种恶性肿瘤, 其发病的分子机制目前尚不清楚. 人体细胞错配修复(mismatch repair MMR)系统的主要功能是修复DNA复制时形成的错误, 维持人体基因组的稳定性[1-3]. 当MMR的功能障碍时DNA复制时形成的错误因得不到修复而在细胞内积累[4-5]. 基因突变的积累则是多种肿瘤发生的分子基础. 细胞错配修复功能的丧失是欧美多见的遗传性非息肉性大肠癌(hereditery nonpolyposis colorectal carcinoma, HNPCC)发病的主要原因之一[6-7]. 我国常见的胃癌的发生是否也与错配修复功能缺陷有关? 我们分析胃癌微卫星不稳定性与错配修复蛋白hMLH1和hMSH2表达的关系, 对这一问题进行探讨.

1 材料和方法

1.1 材料 胃癌及癌旁组织标本56例取自中国东北地区临床外科手术患者, 年龄28-71(平均57岁), 男32例, 女24例, 其中早期癌20例, 中晚期癌36例, 高分化癌22例, 中低分化癌34例, 手术标本部分用石蜡包埋, 部分于-80 ℃保存, 备用. 用常规酚-氯仿法提取组织DNA. Perkin-Elemer Model 2700 PCR扩增仪、ABI Prism 310基因分析仪、商品化分离胶(美国ABI公司); P/ACE MDQ型毛细管电泳(美国Beckman公司); 丙烯酰胺、四甲基乙二胺(Temed)、过硫酸胺(APS)、γ-甲基丙烯酰基-三(甲氧基)硅烷与TBE电泳缓冲液(Sigma), PCR反应试剂、引物(宝生物工程公司), 抗hMSH2小鼠mAb(美国Calbio Chem公司), 抗hMLH1小鼠mAb(美国Pharm Ingen公司), 免疫组化试剂盒(北京中山生物技术有限公司).

1.2 方法 选取五个微卫星位点, 引物设计参考文献[8], 序列(见表1). PCR反应体积12.5 μL, 其中模板DNA 50 ng, 4 × dNTPs终浓度各0.2 mmoL/L, $MgCl_2$的浓度1.5 mmoL/L, Taq酶0.5 U. 循环条件: 94 ℃预变性5 min后, 94 ℃ 30 s, 58-60 ℃ 30 s, 72 ℃ 30 s, 35个循环周期, 最后72 ℃延长5 min. PCR产物各取0.5 μL, 加入1 μL GeneScan 500 size standard和12 μL水, 然后95 ℃变性5 min后迅速冰浴冷却, 待测. 电泳时由毛细管电泳仪压力系统自动填充筛分递质到毛细管中(总长47 cm, 有效长度36 cm, 内径50 μm), 60 g/L的SLPA添加8 moL/L尿素做筛分递质, 分析温度60 ℃, 分离电压15 kV, 激光诱导荧光检测器检测DNA片段, GeneScan数据处理软件采集数据. 被检测的五个微卫星位点如出现两个或两个以上位点的不稳定, 定为微卫星的高度不稳定(MSI-H), 一个位点出现不稳定定为微卫星的低度不稳定(MSI-L), 没有位点出现不稳定定为微卫星稳定(MS-S). 石蜡切片常规免疫组织化学SP方法检测hMLH1和hMSH2蛋白的表达, 厚度为4 μm, hMLH1mAb稀释度为1∶60, hMSH2mAb稀释度为1∶80, 37 ℃温育1 h. 以后步骤均按试剂盒说明书进行, 用DAB呈色. 肿瘤组织上皮细胞核不着色, 而周围组织上皮细胞核着色判定为hMLH1或hMSH2蛋白表达的缺失.

表1 微卫星位点的引物序列

位点	引物序列	退火温度(℃)
BAT-26	5'-FAM- TGACTACTTTTGACTTCAGCC	58
	5'-AACCATTCAACATTTTTAACCC	
D17S261	5'-HEX-AGGGATACTATTCAGCCCGAGGTG	60
	5'-ACTGCCACTCCTTGCCCCATTC	
D3S1283	5'-TET-GGCAGTACCACCTGTAGAAATG	60
	5'-GAGTAACAGAGGCATCGTGTATTC	
D2S123	5'-FAM-AAACAGGATGCCTGCCTTTA	60
	5'-GGACTTTCCACCTATGGGAC	
D3S1611	5'-HEX-CCCCAAGGCTGCACTT	60
	5'-AGCTGAGACTACAGGCATTTG	

2 结果

胃癌56例中, 微卫星高度不稳定14例, 低度不稳定

4例，稳定38例；由于微卫星低度不稳定病例不足5例，统计时与微卫星稳定病例合并. 据此，将56例胃癌分为二组：微卫星不稳定组(MSI)14例(25%)和微卫星稳定组(MSS)42例(75%). 胃癌组织中，14例(25%)表现出错配修复蛋白表达缺失. 14例微卫星不稳定的胃癌组织中，11例(79%)有hMLH1蛋白或hMSH2蛋白表达的缺失，42例微卫星稳定的胃癌组织仅3例(7%)有hMLH1或hMSH2表达的缺失(表2)，胃癌的微卫星不稳定与错配修复蛋白表达的缺失高度相关($P<0.01$). 图1所示5个微卫星序列在相同电泳条件下的电泳图谱；图2所示胃癌组织hMLH1和hMSH2蛋白表达的免疫组织化学染色.

表2 胃癌微卫星不稳定性与错配修复蛋白表达缺失的比较

微卫星位点	MMR表达缺失(n)		合计
	hMLH1	hMSH2	
MSI(14例)	10	3	79% (11/14)[a]
MSS(42例)	2	1	7% (3/42)

MSI-微卫星不稳定，MSS-微卫星稳定，MMR-错配修复；[a]MSI组hMSH2表达缺失的3例胃癌有2例伴随hMLH1表达的缺失，MSI与MSS组MMR蛋白表达缺失率比较$\chi^2=28.57$，$P<0.01$.

根据临床分级和分期对56例胃癌的微卫星不稳定性和错配修复蛋白表达缺失进行分析(表3)，结果进展期胃癌的微卫星不稳定性明显高于早期胃癌，而在不同分化程度的胃癌之间微卫星不稳定性无明显差异；错配修复蛋白表达缺失在不同的分化程度和临床分期的胃癌差异不明显.

表3 胃癌微卫星不稳定和MMR表达缺失与临床分级、分期的分析

分组	MSI		MMR表达缺失	
	n	P值	n	P值
高分化 (22)	7 (32%)	0.50	6 (27%)	0.75
中低分化 (34)	7 (21%)		8 (24%)	
早期 (20)	1 (5%)	0.01[a]	3 (15%)	0.33
中晚期 (36)	13 (36%)		11 (31%)	

MSI-微卫星不稳定，MMR-错配修复；[a]微卫星不稳定性在中晚期胃癌明显高于早期胃癌($\chi^2=6.64$，$P<0.05$)；不同分化程度的胃癌微卫星不稳定性差异不明显，高分化与低分化以及早期与中晚期的胃癌MMR蛋白表达缺失均无显著性差异.

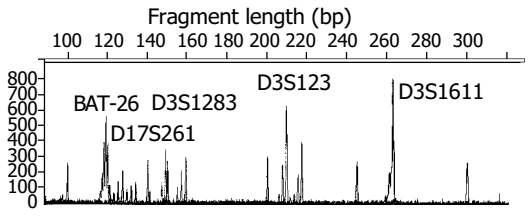

图1 5个微卫星序列的电泳图. (红色峰是GeneScan 500 size标准峰) 电泳条件：毛细管47cm × 50 μm内径；缓冲液1 × TBE内含8 moL/L尿素；分离递质60 g/L SLPA+8 moL/L尿素；进样量15 kV × 5 s；分离温度60 ℃；分离电压-15 kV. 在该条件下，可以对5个微卫星位点同时进行检测.

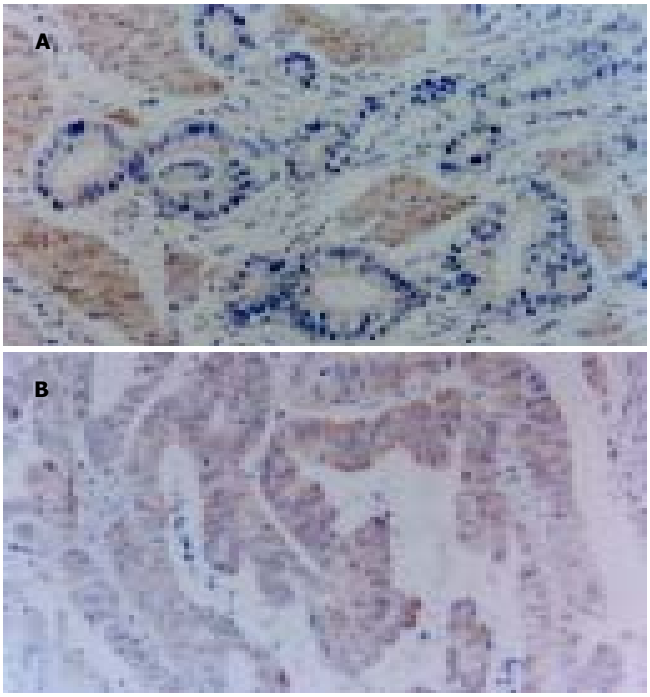

图2 胃癌组织hMLH1和hMSH2蛋白表达的免疫组化染色. A：浸润到肌层的胃癌组织hMLH1蛋白表达的缺失：癌组织细胞核不着色，而癌周围组织细胞核着色；B：hMSH2表达阳性的胃癌细胞核的着色 (放大倍数 × 200).

3 讨论

微卫星是由1-6个碱基组成的简单重复序列，广泛分布于人类基因组内[9-10]. DNA复制时，微卫星序列很容易形成错配或重组错误. 当细胞的错配修复系统功能障碍时，这些错误得不到修复，微卫星表现为重复单位的增加或减少，称为微卫星不稳定性(microsatellite instability，MSI)[11-12]. 15%的大肠癌具有遗传背景，HNPCC的发生率占全部结肠癌的0.5-13%[13-15]. 有报告90%的HNPCC的肿瘤细胞中都能检测到MSI，且影响MSI的主要是单个和二个碱基的重复序列[16-19]. 我们在本实验中选取的微卫星位点也是由单个和二个碱基的重复序列，运用聚丙烯酰胺毛细管电泳在25%的胃癌组织中检测到了MSI，这与大肠癌相似，可以认为这些胃癌的发生与胃黏膜细胞的错配修复功能缺陷有关. 而另一部分胃癌的发生可能与错配修复功能缺陷无关. 这提示多种不同机制可能参与胃癌发生的过程.

HMLH1和hMSH2基因是错配修复基因家族中的重要成员，其编码的蛋白质作为错配修复体系中的起连接和识别作用的亚基，在错配修复过程中起重要作用. 在HNPCC，由于这两种基因的突变或蛋白表达的异常而造成的细胞错配修复功能障碍的占80%以上[20-24]. 到目前为止，已经检测到400多种错配修复基因的突变，MLH1约占50%，MSH2约占40%(国际合作组织关于HNPCC网站：http/www.nfdht.nl)[25-28]. 我们在25%的胃癌组织中检测到错配修复基因hMLH1或hMSH2蛋白表达的缺失，这与MSI的出现率恰好吻合. MSI的胃癌中有79%出现MMR蛋白表达的缺失，我们可以推测由于hMLH1和hMSH2

(主要是hMLH1)蛋白缺失而导致的细胞错配修复功能缺陷在MSI的胃癌的发生过程中起重要作用.

对MSI和MMR表达缺失的胃癌根据不同分化程度和不同临床分期进行进一步的分析发现: MSI随着胃癌的演进而增加,而与胃癌的分化程度关系不明显; MMR的表达缺失与胃癌的生物学行为无关.这从另一个侧面说明, 与结肠癌一样, 细胞MMR缺陷是胃癌发生的早期事件, 也就是说, 胃黏膜细胞MMR缺陷导致了MSI以及基因组的不稳定, 而基因组的不稳定使胃黏膜细胞恶变的危险性增高.反过来, 胃癌在演进过程中也促进了基因组的不稳定性的累积.

典型的HNPCC体细胞内存在MLH1和MSH2的胚系突变, 当携带一条等位基因已发生突变的体细胞受到第二次打击时, 错配修复功能即丧失. 至于胃癌的MMR缺失是否同HNPCC一样来源于遗传, 或者说存在着遗传性胃癌, 还有待于探讨.

4 参考文献

1 Jacob S, Praz F. DNA mismatch repair defects: role in colorectal carcinogenesis. *J Biochimie* 2002;84:27-47

2 Palombo F, Iaccarino I, Nakajima E, Ikejima M, Shimada T, Jiricny J. hMutSbeta, a heterodimer of hMSH2 and hMSH3, binds to insertion / deletion loops in DNA. *Curr Biol* 1996;6:1181-1184

3 Genschel J, Littman SJ, Drummond JT, Modrich P. Isolation of MutSbeta from human cells and comparison of the mismatch repair specificities of MutSbeta and MutSalpha. *J Biol Chem* 1998;273:19895-19901

4 Coleman WB, Tsongalis GJ. The role of genomic instability in human carcinogenesis. *Anticancer Res* 1999;19:4645-4664

5 Duval A, Hamelin R. Genetic instability in human mismatch repair deficient cancers. *Ann Genet* 2002;45:71-75

6 Cui L, Jin HY, Cheng HY, Yan YD, Meng RG, Yu DH. Genetic detection of Chinese hereditary nonpolyposis colorectal cancer. *World J Gastroenterol* 2004;10:209-213

7 Salovaara R, Loukola A, Kristo P, Kaariainen H, Ahtola H, Eskelinen M, Harkonen N, Julkunen R, Kangas E, Ojala S, Tulikoura J, Valkamo E, Jarvinen H, Mecklin JP, Aaltonen LA, de la Chapelle A. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2000; 18:2193-2200

8 Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J. Diagnostic microsatellite instability:definition and correlation with mismatch repair protein expression. *Cancer Res* 1997;57:4749-4756

9 Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993;260:812-816

10 Bowcock A, Osborne-Lawrence S, Barnes R, Chakravarti A, Washington S, Dunn C. Microsatellite polymorphism linkage map of human chromosome 13q. *Genomics* 1993;15:376-386

11 Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition:development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;58:5248-5257

12 Loukola A, Eklin K, Laiho P, Salovaara R, Kristo P, Jarvinen H, Mecklin JP, Launonen V, Aaltonen LA. Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC). *Cancer Res* 2001;61:4545-4549

13 Cunningham JM, Kim CY, Christensen ER, Tester DJ, Parc Y, Burgart LJ, Halling KC, McDonnell SK, Schaid DJ, Walsh Vockley C, Kubly V, Nelson H, Michels VV, Thibodeau SN. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. *Am J Hum Genet* 2001; 69:780-790

14 Samowitz WS, Curtin K, Lin HH, Robertson MA, Schaffer D, Nichols M, Gruenthal K, Leppert MF, Slattery ML. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. *Gastroenterology* 2001;121:830-838

15 Percesepe A, Borghi F, Menigatti M, Losi L, Foroni M, Di Gregorio C, Rossi G, Pedroni M, Sala E, Vaccina F, Roncucci L, Benatti P, Viel A, Genuardi M, Marra G, Kristo P, Peltomaki P, Ponz de Leon M. Molecular screening for hereditary nonpolyposis colorectal cancer: A prospective, population-based study. *J Clin Oncol* 2001;19:3944-3950

16 Terdiman JP, Gum JR Jr, Conrad PG, Miller GA, Weinberg V, Crawley SC, Levin TR, Reeves C, Schmitt A, Hepburn M, Sleisenger MH, Kim YS. Efficient detection of hereditary nonpolyposis colorectal cancer gene carriers by screening for tumor microsatellite instability before germline genetic testing. *Gastroenterology* 2001;120:21-30

17 Liu T, Wahlberg S, Burek E, Lindblom P, Rubio C, Lindblom A. Microsatellite instability as a predictor of a mutation in a DNA mismatch repair gene in familial colorectal cancer. *Genes Chromosomes Cancer* 2000;27:17-25

18 Potocnik U, Glavac D, Golouh R, Ravnik-Glavac M. Evaluation of microsatellite markers for efficient assessment of high microsatellite instabile colorectal tumors. *Pflugers Arch* 2000;439:47-49

19 Percesepe A, Kristo P, Aaltonen LA, Ponz de Leon M, de la Chapelle A, Peltomaki P. Mismatch repair genes and mononucleotide tracts as mutation targets in colorectal tumors with different degrees of microsatellite instability. *Oncogene* 1998;17:157-163

20 Harfe BD, Jinks Robintson S. DNA mismatch repair and genetic instability. *Annu Rev Genet* 2000;34:359-399

21 Peltomaki P, Vasen HF. Mutations predisposing to hereditary nonpolyposis colorectal cancer: Database and results of a collaborative study. The international collaborative group on HNPCC. *Gastroenterology* 1997;113:1146-1158

22 Vasen HF, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, Nagengast FM, Meijers-Heijboer EH, Bertario L, Varesco L, Bisgaard ML, Mohr J, Fodde R, Khan PM. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. *Gastroenterology* 1996;110:1020-1027

23 Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, Peltomaki P, Mecklin JP, Jarvinen HJ. Cancer risk in mutation carriers of DNA mismatch repair genes. *Int J Cancer* 1999;81:214-218

24 Kruse R, Rutten A, Lamberti C, Hosseiny-Malayeri HR, Wang Y, Ruelfs C, Jungck M, Mathiak M, Ruzicka T, Hartschuh W, Bisceglia M, Friedl W, Propping P. Muir-Torre phenotype has a frequency of DNA mismatch-repair-gene mutations similar to that inhereditary nonpolyposis colorectal cancer families defined by the Amsterdam criteria. *Am J Hum Genet* 1998;63:63-70

25 Dieumegard B, Grandjouan S, Sabourin JC, Le Bihan ML, Lefrere I, Bellefqih, Pignon JP, Rougier P, Lasser P, Benard J, Couturier D, Bressac-de Paillerets B. Extensive molecular screening for hereditary nonpolyposis colorectal cancer. *Br J Cancer* 2000;82:871-880

26 Moslein G, Tester DJ, Lindor NM, Honchel R, Cunningham JM, French AJ, Halling KC, Schwab M, Goretzki P, Thibodeau SN. Microsatellite instability and mutation analysis of hMSH2and hMLH1 in patients with sporadic, familial and hereditary colorectal cancer. *Hum Mol Genet* 1996;5:1245-1252

27 Bapat BV, Madlensky L, Temple LK, Hiruki T, Redston M, Baron DL, Xia L, Marcus VA, Soravia C, Mitri A, Shen W, Gryfe R, Berk T, Chodirker BN, Cohen Z, Gallinger S. Family history characteristics, tumor microsatellite instability and germlineMSH2 and MLH1 mutations in hereditary colorectal cancer. *Hum Genet* 1999;104:167-176

28 Nystrom-Lahti M, Holmberg M, Fidalgo P, Salovaara R, de la Chapelle A, Jiricny J, Peltomaki P. Missense and nonsense mutations in codon 659 of MLH1 cause aberrant splicing of messengerRNA in HNPCC kindreds. *Genes Chromosomes Cancer* 1999;26:372-375