•研究快报 BRIEF REPORT •

MMP-2 和 MMP-9 蛋白在结肠癌中的表达

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Expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 protein in colonic carcinoma

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Abstract

AIM: To analyze the correlation of matrix metalloproteinase-2(MMP-2) and MMP-9 protein expression with the pathological factors in colonic carcinoma, and to investigate the clinical significance of matrix metalloproteinases in the occurrence of colonic carcinoma.

METHODS: The expression of MMP-2 and MMP-9 protein were measured by immunohistochemistry in the resected specimens from 31 patients with colonic carcinoma, and their relations with clinicopathologic factors were analyzed by SPSS 10.0 for Windows software. The specimens from ulcerative colitis (n = 20), colon adenoma (n = 21), and normal colon (n = 10) tissues were used as the controls.

RESULTS: The positive rate of MMP-2 protein expression was significantly different between colonic carcinoma and normal colon tissues (10.0% *vs* 54.8%, P <0.05), while no significant difference was found between other groups. The positive rates of MMP-2 and MMP-9 expression tended to increase in ulcerative colitis, colon adenoma, and colonic carcinoma gradually. The positive rates of MMP-2 and MMP-9 protein expression were significantly correlated with the Dukes's staging and lymph node metastasis (C-D stage *vs* A-B stage: 76.9% *vs* 38.9%, P <0.05; 84.6% *vs* 27.8%, P <0.05; metastasis *vs* non-metastasis: 76.9% *vs* 38.9%, P <0.05; 84.6% *vs* 27.8%, P <0.05).

CONCLUSION: Over-expression of MMP-2 and MMP-9 protein play important roles in the carcinogenesis and metastasis of colonic carcinoma.

Key Words: Colonic carcinoma; Matrix metalloproteinases; Ulcerative colitis; Colonic adenoma

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摘要

目的: 分析基质金属蛋白酶-2(MMP-2)和MMP-9蛋白与结肠癌病理因素的关系, 探讨MMP蛋白在结肠癌发生中的临床意义.

方法: 用SP免疫组化法检测31例结肠癌中MMP-2和MMP-9蛋白表达情况, 并用SPSS 10.0 for Windows软件统计分析MMP蛋白与临床病理因素的关系. 以20例溃疡性结肠炎、21例结肠腺瘤和10例正常结肠黏膜作为对照组.

结果: MMP-2除结肠癌组和正常结肠黏膜组间相比具有显著性差异(10.0% *vs* 54.8%, P<0.05)外, 其余各组间比较差异无统计学意义, MMP-9各组间比较差异无统计学意义, 从溃疡性结肠炎、结肠腺瘤到结肠癌中MMP-2和MMP-9蛋白表达阳性率不同且具有递增趋势. MMP-2和MMP-9蛋白表达与结肠癌的Duke's分期及有无淋巴结转移显著相关(A+B期: 38.9%和27.8%; C+D期: 76.9%和84.6%; 无淋巴结转移: 38.9%和27.8%; 有淋巴结转移: 76.9%和84.6%, P<0.05).

结论: MMP-2和MMP-9蛋白过度表达对结肠癌的诊断、Duke's分期及有无淋巴结转移判断具有重要的临床意义.

关键词: 结肠肿瘤; 基质金属蛋白酶类; 溃疡性结肠炎; 结肠腺瘤

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0 引言

肿瘤在生长和转移过程中必须破坏由细胞间基质和基底膜组成的细胞外基质(ECM)[1]. 目前研究证明, 基质

金属蛋白酶类(matrix metalloproteinases, MMPs)对肿瘤的侵袭和转移起着极为重要的作用[2-5]. MMPs中主要是MMP-2、MMP-9参与ECM降解. 我们应用免疫组织化学方法研究MMP-2和MMP-9蛋白等在溃疡性结肠炎、结肠腺瘤、结肠癌等过程中的不同表达, 并分析MMP-2和MMP-9蛋白与结肠癌的病理因素关系, 探讨MMP-2和MMP-9蛋白在结肠癌发生中的作用.

1 材料和方法

1.1 材料 随机选取马鞍山市人民医院1998-2003经手术病理证实的结肠癌31例, 登录各个患者的年龄、性别, 并根据临床和病理资料对于肿瘤的部位、组织学类型、肿瘤大小、有无转移、Duke's分期及浸润深度各项资料进行分析. 31例结肠癌中男性11例, 女性20例, 年龄40-73岁, 平均61.9±8.7岁; <60岁10例, ≥60岁21例; 位于右半结肠11例, 横结肠2例, 左半结肠18例; 肿瘤直径<5 cm 13例, ≥5 cm 18例; A期4例, B期14例, C期11例, D期2例; 管状腺癌25例, 乳头状腺癌1例, 黏液腺癌4例, 未分化腺癌1例; 无淋巴结转移18例, 有淋巴结转移13例; 浸润至黏膜下层1例, 深肌层3例, 浆膜层27例. 另随机收集20例溃疡性结肠炎、21例结肠腺瘤和10例正常结肠黏膜作为对照组, 标本取自马鞍山市人民医院1998-2003的存档蜡块. 鼠抗人MMP-2、MMP-9多克隆抗体购自Santa Cruz Biotechnology公司, SP免疫组化试剂盒均购自福州迈新公司.

1.2 方法 对31例结肠癌的病理蜡块, 重新制作石蜡切片, 厚4 μm, 用3 mL/L H_2O_2灭活内源性过氧化物酶10 min. PBS(pH 7.4)水洗. 然后分别与一抗(鼠抗人MMP-2 1∶100稀释; 鼠抗人MMP-9 1∶150稀释)、二抗及过氧化物酶标记的链霉素亲和素抗体孵育, 以PBS代替一抗作为阴性对照. PBS水洗后DAB显色15 min, 苏木素复染, 封片观察.

免疫组化结果判定[6]: MMP-2, MMP-9染色以细胞质出现棕黄色颗粒为阳性. 阳性细胞数: 0分为无阳性细胞, 1分为阳性细胞数<25%, 2分为阳性细胞25-50%, 3分为阳性细胞51-75%, 4分为阳性细胞数>75%. 染色强度: 0分为无染色, 1分为弱染色, 2分为中等强度染色, 3分为强染色. 计算各抗体的阳性细胞数和染色强度的得分, 以3-7分为阳性.

统计学处理 百分率比较用χ^2检验, 所有统计处理均在SPSS 10.0统计软件包中完成.

2 结果

2.1 正常结肠组织、溃疡性结肠炎、结肠腺瘤和结肠癌中MMP-2, MMP-9蛋白的表达率比较 MMP-2和MMP-9染色阳性物质主要分布在结肠癌细胞的细胞质, 呈散在或团状分布, 部分间质细胞如血管内皮细胞、纤维母细胞中亦有MMP-2, MMP-9表达, 但表达强度弱于癌细胞. 在结肠腺瘤、溃疡性结肠炎以及正常结肠黏膜组织中的阳性染色细胞主要位于结肠腺体部位.

结肠癌中MMP-2, MMP-9的阳性表达率分别为54.8%和58.1%(表1), 正常黏膜组织中阳性表达率分别为10.0%和30.0%, 以弱或中等表达为主. 结肠腺瘤中MMP-2, MMP-9阳性表达率分别为42.9%和52.4%, 溃疡性结肠炎中MMP-2, MMP-9阳性表达率分别为30.0%和40.0%, 均以强表达为主.

表1 正常结肠组织、溃疡性结肠炎、结肠腺瘤和结肠癌中MMP-2, MMP-9蛋白的表达率比较

组织学类型	*n*	MMP-2		阳性率	*P*值	MMP-9		阳性率	*P*值
		−	+	(%)		−	+	(%)	
正常结肠组织	10	9	1	10.0		7	3	30.0	
溃疡性结肠炎	20	14	6	30.0	>0.05	12	8	40.0	>0.05
结肠腺瘤	21	12	9	42.9	>0.05	10	11	52.4	>0.05
结肠癌	31	14	17	54.8	<0.05[a]	13	18	58.1	>0.05

[a]$P<0.05$ *vs* 对照组.

在结肠组织MMP的表达中, MMP-2除结肠癌组和正常结肠黏膜组间相比具有显著性差异($P<0.05$)外, 其余各组间比较差异无统计学意义. MMP-9各组间比较差异无统计学意义. 但从正常结肠黏膜组织、溃疡性结肠炎、结肠腺瘤到结肠癌的MMP-2, MMP-9表达具有递增趋势.

2.2 结肠癌中MMP-2, MMP-9蛋白表达与临床病理因素的关系 结肠癌中MMP-2和MMP-9蛋白表达水平与患者性别、年龄、肿瘤位置、肿瘤直径、结肠癌分化程度及组织浸润程度无关($P>0.05$). Dukes分期A+B期和C+D期两组中, MMP-2阳性表达率分别为38.9%和76.9%, MMP-9阳性表达率分别为27.8%和84.6%, 组间比较有显著差异($P<0.05$). 结肠癌有淋巴结转移和无淋巴结转移两组中, MMP-2阳性表达率分别为76.9%和38.9%, MMP-9阳性表达率分别为84.6%和27.8%, 组间比较有显著差异($P<0.05$)(表2).

3 讨论

肿瘤侵袭和转移过程涉及肿瘤细胞穿过细胞外基质屏障、血管壁的基底膜及穿出血管壁进入宿主微环境的过程. 细胞外基质是细胞生存的重要内环境, 由胶原、糖蛋白、蛋白多糖等构成, 含有大量蛋白酶、细胞因子、黏附分子. 研究表明, MMP是降解细胞外基质最重要的一组蛋白酶, 在消化系肿瘤的发生、发展及侵袭和转移中具有重要作用[2-5,7]. MMP家族按结构和基质特性不同可分为胶原酶(MMP-1, MMP-8, MMP-13, MMP-18)、明胶酶(MMP-2, MMP-9)、基质降解素(MMP-3, MMP-10, MMP-11)、膜型MMP等4大类[8-10]. 目前发现MMP-2、MMP-9被认为是在肿瘤侵袭转移过程中最直接和最重要的MMP.

本组实验结果显示, MMP-2表达在结肠癌中最高,

表 2 MMP-2，MMP-9表达与结肠癌临床病理因素之间的关系

变量		MMP-2		阳性率(%)	*P*值	MMP-9		阳性率(%)	*P*值
		−	+			−	+		
性别	男	6	5	45.5	>0.05	7	4	36.4	>0.05
	女	8	12	60.0		8	12	60.0	
年龄(岁)	<60	6	4	40.0	>0.05	6	4	40.0	>0.05
	≥60	8	13	61.9		9	12	57.1	
肿瘤位置	右半结肠	5	4	44.4	>0.05	5	4	44.4	>0.05
	横结肠	1	3	75.0		2	2	50.0	
	左半结肠	8	10	55.6		8	10	55.6	
肿瘤直径	<5 cm	6	7	53.8	>0.05	8	5	38.5	>0.05
	≥5 cm	8	10	55.6		7	11	61.1	
Dukes分期	A+B期	11	7	38.9	<0.05	13	5	27.8	<0.05
	C+D期	3	10	76.9		2	11	84.6	
组织学类型	管状腺癌	11	14	56.0	>0.05	11	14	56.0	>0.05
	乳头状腺癌	0	1	100.0		1	0	0.0	
	黏液腺癌	2	2	50.0		2	2	50.0	
	未分化癌	1	0	0.0		1	0	0.0	
淋巴结转移	无	11	7	38.9	<0.05	13	5	27.8	<0.05
	有	3	10	76.9		2	11	84.6	
组织浸润程度	黏膜下或肌层	3	1	25.0	>0.05	3	1	25.0	>0.05
	浆膜或浆膜外层	11	16	59.3		12	15	55.6	

MMP-9蛋白表达在结肠癌中较高, 但差异无显著性, 其次为结肠腺瘤和溃疡性结肠炎, 正常结肠黏膜组织中最低, 但各组间比较无显著性差异, MMP-2、MMP-9在不同组织类型中表达符合溃疡性结肠炎→结肠腺瘤→结肠癌的演变趋势. Levy *et al*[11-13]报道有72%的结直肠腺癌组织中的MMP-2 mRNA水平显著升高, 且阳性率与肿瘤的浸润深度与转移相关. 黄文斌 *et al*[14]采用免疫组化法检测87例结肠癌组织中MMP-2表达情况, 结果发现结肠癌组织中MMP-2侵及结肠肌层的阳性表达率明显低于侵及结肠浆膜层者, MMP-2的表达与肿瘤的侵袭、转移有关. 同时相关研究结果提示, MMP-2、MMP-9表达与溃疡性结肠炎的结肠炎症程度相关, MMP-2、MMP-9参与溃疡性结肠炎患者溃疡边缘基底膜的破坏和重塑过程, 抑制MMP-2和MMP-9可有效减轻组织破坏以及结肠炎症过程.

本研究结果显示, 结肠癌中MMP-2和MMP-9蛋白表达水平与患者性别、年龄、肿瘤位置、肿瘤直径、结肠癌分化程度及组织浸润程度无关. 在有淋巴结转移的结肠癌中MMP-2、MMP-9表达率明显高于无淋巴结转移者, 组间比较有显著差异, 提示结肠癌组织中MMP-2和MMP-9通过降解细胞外基质, 促进结肠肿瘤细胞向肠壁深层浸润, 发生淋巴结转移. Dukes分期中C+D期的结肠癌中MMP-2、MMP-9表达率明显高于A+B期, 组间比较有显著差异, 提示结肠癌中MMP-2、MMP-9过度表达与结肠癌Dukes分期和结肠癌的侵袭性密切相关. 本研究结果与Baker *et al*[15-18]研究结果一致, 表明MMP-2、MMP-9在促进结肠癌的淋巴结转移过程中起重要作用, 具体机制尚待进一步研究.

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•研究快报 BRIEF REPORT •

直肠癌 TME 术后检测癌组织及直肠系膜内 MMP–7 的意义

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Significance of detection for matrix metalloproteinase-7 in rectal cancer tissues and mesorectum after total mesorectal excision

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Abstract

AIM: To detect the expression of matrix metalloproteinase(MMP)-7 in the mesorectum of patients with rectal cancer, and to provide theoretical evidence for the total mesorectal excision (TME).

METHODS: The expression of MMP-7 was detected in the specimens from cancer tissues, mesorectum (in the plane, tissues 2 cm distal to the lower margin, and the distal end of the tumor), and outer pelvic fascia by SP immunohistochemistry in 47 patients after TME. And the data were compared with the result of HE staining.

RESULTS: The positive rate of MMP-7 expression was 29.8% (14/47) in the mesorectum. Of those positive expressions, 7 were in the plane of tumor, 3 in both the tumor plane and tissues 2 cm distal to the lower margin of the tumor, and 4 only in the tissues 2 cm distal to the tumor. The positive rate was 14.8% (7/47) by HE staining, and the result of MMP-7 staining was all positive. The positive rate of MMP-7 expression was 91.5% (14/47) in the tumor tissues. There was no MMP-7 expression in the mesorectum, distal end of the outer pelvic fascia, and the control tissues (normal