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电编 张敏 编辑 张海宁

ISSN 1009-3079 CN 14-1260/R 

•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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收稿日期: 2005–08–29 接受日期: 2005–09–06

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

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Received: 2005-08-29 Accepted: 2005-09-06

## 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

rectal mucosa and mesorectum 10 cm distal to the upper margin of the tumor).

**CONCLUSION:** MMP-7 is expressed in mesorectal tissues of the tumor plane and tissues 2 cm distal to the tumor. The positive expression of MMP-7 is correlated with the degree of the tumor differentiation, depth of invasion, and the Dukes staging.

Key Words: Rectal cancer; Mesorectum; Matrix metalloproteinase-7

Zhang HG, Tian SL, Zhang RP. Significance of detection for matrix metalloproteinase-7 in rectal cancer tissues and mesorectum after total mesorectal excision. Shijie Huaren Xiaohua Zazhi 2005;13(20):2497-2500

**摘要**

**目的:** 研究直肠癌患者直肠系膜内MMP-7表达, 为全直肠系膜切除术(total mesorectal excision, TME)提供理论依据.

**方法:** 直肠癌TME术后, 应用免疫组化SP法检测47例患者癌组织、直肠系膜(肿瘤平面、肿瘤下缘下2 cm和系膜远端)及盆筋膜壁层MMP-7的表达. 并与病理组织学染色(常规HE染色)结果进行比较.

**结果:** 直肠系膜中MMP-7的阳性表达率为29.8%(14/47), 有7例患者只在肿瘤平面系膜内有MMP-7表达, 3例在肿瘤平面和肿瘤下缘下2 cm处系膜内有MMP-7表达, 4例只在肿瘤下缘下2 cm有MMP-7阳性表达. 常规病理染色阳性率为14.8%(7/47), 其MMP-7染色均为阳性. 在癌组织中, MMP-7的阳性率为91.5%(14/47). 在直肠系膜远端、盆筋膜壁层和对照组织(距肿瘤上缘10 cm以上的正常直肠黏膜和系膜)中, MMP-7均无表达.

**结论:** 直肠系膜内MMP-7表达可及肿瘤平面和肿瘤下缘下2 cm处, 但尚未达到直肠系膜远端和盆筋膜壁层. 肿瘤的分化程度、肠壁浸润深度和Dukes分期与系膜内MMP-7阳性表达有关. 从分子病理学的角度可以认为TME是科学的.

**关键词:** 直肠癌; 直肠系膜; MMP-7

张好刚, 田素礼, 张曰平. 直肠癌TME术后检测癌组织及直肠系膜内MMP-7的意义. 世界华人消化杂志 2005;13(20):2497-2500
http://www.wjgnet.com/1009-3079/13/2497.asp

## 0 引言

基质金属蛋白酶-7(matrix metalloproteinase-7, MMP-7)是一种与肿瘤浸润和转移密切相关的蛋白水解酶[1]. 在直肠癌中, 只在癌细胞和个别异形性上皮细胞表达, 在肿瘤间质和淋巴细胞中均不表达, 具有较强的癌细胞特异性, 是一种肿瘤微转移的标志物[2-5].

我们在全直肠系膜切除术(total mesorectal excision, TME)术后, 检测癌组织、直肠系膜和盆筋膜壁层组织中MMP-7的表达, 希望能够了解直肠系膜内MMP-7的表达特点, 从分子病理学的角度为TME提供理论依据.

## 1 材料和方法

1.1 材料 选取2004-04/2005-04的直肠癌患者47例, 所有病例均经病理证实. 其中男性28例、女性19例, 年龄48-79岁, 中位年龄59岁. Dukes分期A期19例、B期15例、C期13例. 47例患者均行TME, 其中Dixon术37例, Miles术10例.

1.2 方法

1.2.1 标本采集过程 (1)手术中, 在肿瘤平面和距离肿瘤下缘2 cm处的盆筋膜壁层各取一块组织(大小1.0 cm×1.0 cm). (2)手术结束时, 立即将新鲜切除的手术标本平铺在无菌单上, 沿直肠远切缘游离直肠系膜至肿瘤下缘. 无张力下测量系膜长度. 在肿瘤下缘、肿瘤下缘下2 cm和直肠系膜远端分别截取1 cm宽的直肠系膜. (3)在肿瘤对侧纵向剖开肠管, 无菌生理盐水洗净, 避开缺血及坏死区, 在肿瘤实质取1.0 cm×1.0 cm大小组织2块. 将切取组织置入40 g/L甲醛溶液中固定. 同时测量肿瘤大小, 观察大体分型, 记录患者临床资料. 另采集因直肠良性病变, 行直肠部分切除的标本15例, 取正常直肠黏膜及正常直肠系膜作为对照组.

1.2.2 染色 将所切取标本常规石蜡包埋, 每隔1 mm连续切片2张, 切片厚5 μm. 将每2张切片随机分配, 其中一张行常规病理染色(发现微转移灶为阳性), 另一张行免疫组化MMP-7染色(抗MMP-7抗体和UltraSensitive™ S-P超敏试剂盒购自福州迈新公司). 以迈新公司提供阳性片为阳性对照, PBS代替一抗作为阴性对照. 细胞膜或胞质染成棕黄色、棕褐色颗粒为阳性, 阴性细胞不着色. 高倍镜下(×400)观察染色较好的二十个视野, 计数阳性细胞, 1-5个为+, 6-25个为++, 26个以上为+++.

**统计学处理** 应用SPASS 10.0统计软件对数据进行$\chi^2$检验或Fisher精确检验, $P<0.05$有统计学意义.

## 2 结果

2.1 各组织中MMP-7的表达 MMP-7在对照组中无表达, 在直肠癌中阳性率为91.5%(43/47), 在直肠系膜中为29.8%(14/47), 与对照组比较均有显著性差异. 盆筋膜壁层MMP-7无表达. 在直肠系膜中, 有7例患者只在肿瘤平面系膜内有MMP-7表达, 3例在肿瘤平面和肿瘤下缘下2 cm处系膜内有MMP-7表达, 4例只在肿瘤下缘下2 cm有MMP-7阳性表达. 直肠癌直肠系膜MMP-7的表达与肿瘤的临床病理特征的关系(表1).

2.2 常规病理染色和MMP-7免疫组织化学染色阳性表达情况 通过常规病理染色, 我们发现有7例患者的标本在直肠系膜中有转移灶, 阳性率为14.8%(7/47). 这7例患者的

表1 直肠癌临床病理特征与直肠系膜MMP-7的表达的关系

| 临床病理特征 | | n | MMP-7表达 | | | | | P值 |
|---|---|---|---|---|---|---|---|---|
| | | | − | + | ++ | +++ | 阳性率(%) | |
| 肿瘤形态 | 肿块型 | 14 | 11 | 1 | 1 | 0 | 14.2 | |
| | 溃疡型 | 22 | 16 | 2 | 3 | 2 | 31.8 | <0.05 |
| | 浸润型 | 11 | 6 | 0 | 3 | 2 | 45.4 | |
| 分化程度 | 高+中 | 24 | 17 | 2 | 3 | 31 | 16.6 | |
| | 低分化 | 23 | 16 | 3 | 3 | 1 | 30.4 | <0.05 |
| 浸润深度 | $T_1$+ $T_2$ | 22 | 15 | 3 | 1 | 3 | 18.1 | |
| | $T_3$+ $T_4$ | 25 | 18 | 2 | 4 | 1 | 28.0 | <0.05 |
| TNM分期 | I+II | 19 | 5 | 0 | 2 | 1 | 15.7 | |
| | III+IV | 15 | 10 | 3 | 1 | 1 | 33.3 | <0.05 |
| 肿瘤纵径 | ≤3 cm | 20 | 14 | 1 | 3 | 2 | 30.0 | |
| | >3 cm | 19 | 13 | 2 | 3 | 1 | 31.5 | >0.05 |

直肠系膜均有MMP-7表达. 在常规病理染色未发现转移灶的40例患者中, 有7例MMP-7染色阳性, MMP-7染色的阳性率为29.8%(14/47). 两阳性率差别有显著性($P$<0.05).

盆筋膜壁层无常规病理染色阳性, 也无MMP-7阳性表达.

## 3 讨论

手术治疗是直肠癌综合治疗的基础. 近20 a的TME的应用使直肠癌手术治疗效果得到很大的改善. 局部复发率由25%降至3-7%[6-9]. 现在已经广为外科医生们所接受, 是直肠癌外科治疗的金标准. Heald[10-13]和Wang *et al*[14]认为TME应在盆筋膜脏壁两层间锐性切除, 直肠系膜(盆筋膜脏层)完整切除无撕脱, 并保证肉眼及镜下无切缘累及.

然而, 对于肿瘤而言, 其浸润和转移是肿瘤细胞与细胞外基质相互作用的结果. 在其发展成为肉眼及镜下可以检查到的转移灶之前, 已经发生了微转移. 微转移的检测方法有很多种, 连续切片法是最早应用于检测微转移的粗略方法, 而免疫学和分子生物学的发展为检测微小癌灶及少量癌细胞的存在提供了更多特异性高、敏感性强的方法. 在这些方法中免疫组化方法简单、价格便宜, 可以广泛的应用于临床, 并且最重要的是, 它比常规病理组织染色有更高的敏感性[15].

MMP-7是基质金属蛋白酶家族中分子量最小的成员, 具有强大的基质降解活性和广泛的底物特异性, 它在肿瘤的发展、转移和浸润过程中起重要的作用[1]. 在大肠癌中, MMP-7具有很强的癌细胞特异性, 正常黏膜中不表达. 淋巴结中MMP-7的表达可以作为诊断大肠癌淋巴结转移尤其是微转移的依据[16,17]. 我们的结果表明, 正常直肠黏膜和系膜内无MMP-7阳性染色, 91.5%(14/47)的患者癌组织中有MMP-7表达, 与国内外的文献报道相符[18].

在系膜中, 我们通过常规病理染色发现7例微转移, 并且其MMP-7表达均为阳性. 在常规病理染色结果阴性的40例患者中, 有7例MMP-7表达阳性. 这7例患者的直肠系膜中存在微转移灶. 因此TME术后检测直肠系膜中MMP-7的表达能够提高临床分期的准确性, 对判断患者预后、指导术后辅助治疗具有重要意义. 通过直肠癌生物学特征与其系膜中MMP-7表达比较发现, 分化低、浸润深或是Dukes分期晚的直肠癌患者与MMP-7的阳性表达有关. 而肿瘤大小的不同在MMP-7的阳性表达率方面, 我们未发现有差异. 这与肿瘤中MMP-7表达情况并不完全相同.

我们通过比较与肿瘤不同距离的直肠系膜MMP-7表达情况发现, 4例患者只在肿瘤下缘下2 cm有MMP-7阳性表达, 这可能是文献中提到的肿瘤通过静脉和淋巴进行直肠系膜内跳跃式播散的表现[19,20]. 从直肠系膜远端和盆筋膜壁层中我们未检测到MMP-7的表达, 我们可以看出, TME切除微转移灶的彻底性. 这为TME从分子病理学的角度提供了理论依据.

我们只是在纵向研究了直肠系膜的几个平面(肿瘤平面、下缘下2 cm和直肠系膜远端)的MMP-7表达情况, 只能说明这几个纵向平面的情况, 并不能说明MMP-7在系膜内表达具体有多远和多深, 这些尚需要做进一步的研究.

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电编 张敏 编辑 菅鑫妍 审读 张海宁

ISSN 1009-3079 CN 14-1260/R 2005年版权归世界胃肠病学杂志社

•研究快报 BRIEF REPORT •

# 幽门螺杆菌vacA基因重组表达的包涵体复性及ELISA方法的建立

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## Renaturation of *Helicobacter pylori* vacA gene recombined expressive inclusion body and construction of ELISA method

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Received: 2005-08-29 Accepted: 2005-09-06

## Abstract

**AIM:** To increase the biological activity of the expressive product through denaturation and renaturation of *H pylori* vacA gene recombined expressive inclusion body, and to discuss the feasibility of mass production of VacA antigen.

**METHODS:** The VacA antigen expressed engineering bacterium was induced by Isopropyl-β-D-thiogalactopyranoside (IPTG). The expressed inclusion body was denatured, renatured and dialyzed, and its activity was identified. The plates of ELISA were folded by the produced antigen. Then the ELISA method was constructed to determine the biological activity of the