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根除幽门螺杆菌对胃癌前病变组织中bax蛋白表达的影响

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Effect of *Helicobacter pylori* infection on bax protein expression in patients with gastric precancerous lesions

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Abstract

AIM:To evaluate the effect of *Helicobacter pylori* (*Hp*) infection on bax protein expression, and explore the role of *Hp* in the development of gastric carcinoma.

METHODS:*Hp* was examined by rapid urease test and Warthin-Starry method, and bax protein was examined by immunohistochemical staining in 72 patients with pre-malignant lesions.

RESULTS:Bax protein was expressed with different degree in intestinal metaplasia and gastric dysplasia, its positive rate being 63.9%. The positive rate of Bax protein expression in *Hp*-positive gastric precancerous lesions (72.3 %)was significantly higher than that in *Hp*-negative gastric precancerous lesions (48.0 %, χ^2=4.191, P <0.05). *Hp* infection was correlated well with the expression of Bax protein in gastric precancerous lesions(r =0.978, P <0.01). After eradication of *Hp*, the positive rate of bax protein expression was significantly decreased in *Hp*-positive gastric precancerous lesions (χ^2=5.506, P <0.05). In the persistent *Hp* infected patients, the positive rate of Bax protein expression was not changed.

CONCLUSION:*Hp* is involved in the expression of Bax gene. *Hp* infection increases the expression of Bax protein, this may be one of the mechanisms of *Hp* infection in the induction of gastric epithelial cell apoptosis. *Hp* might act as a tumor promoter in the genesis of gastric carcinoma. Eradication of *Hp* could inhibit the formation and development of gastric carcinoma.

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摘要

目的:研究Hp感染及根除治疗对胃癌前病变组织中促凋亡基因Bax蛋白表达的影响,探讨Hp的致病机制及其在胃癌发生中的作用.

方法:采用快速尿素酶试验、Warthin-starry银染色检测Hp;采用免疫组织化学SP法检测72例胃癌前病变组织中Bax蛋白的表达情况,以及Hp阳性患者Hp根除前后胃癌前病变Bax蛋白表达的变化.

结果:Bax蛋白在肠上皮化生及不典型增生组织中均有不同程度的表达,其阳性表达率为63.9 %.Hp阳性胃癌前病变组Bax蛋白阳性表达率(72.3 %)显著高于Hp阴性胃癌前病变组(48.0%,χ^2=4.191,P <0.05),Hp感染与Bax阳性表达及分级呈正相关(r =0.978, P <0.01).经三联治疗根除Hp后,Bax蛋白阳性表达率(70.3 %)较治疗前显著降低(43.2 %,χ^2=5.506, P <0.05),而Hp仍为阳性者Bax蛋白阳性表达率则无变化.

结论:Hp感染可以促进促凋亡基因Bax蛋白的表达,这可能是Hp感染诱导胃黏膜上皮细胞凋亡的主要机制之一,从而导致细胞凋亡和增生的调节紊乱,使胃黏膜上皮不稳定性增加,使其具有较高的癌变易感性.根除Hp感染可纠正Bax基因表达的异常,对预防或减缓胃癌的发生可能具有重要意义.

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0 引言

大量的流行病学资料表明,胃癌发生率与当地的幽门螺杆菌(Helicobacter pylori, Hp)感染率呈正相关,Hp感染者发生胃癌的危险性较非感染者高6倍[1-10].从慢性浅表性胃炎、萎缩性胃炎、肠上皮化生及异型增生到胃癌的演变过程已经明确.Hp感染为慢性胃炎的主要病

因，因此有理由认为 Hp 感染是胃癌发生的危险因素之一[11-18].但其确切的致病机制尚不完全清楚.已有研究表明，Hp 可能通过某些环节造成胃黏膜组织癌基因的突变、细胞信号转导异常等导致胃黏膜细胞发生癌前变化.但通过研究胃癌前病变组织中 Hp 感染及根除治疗对Bax蛋白表达的影响来探讨Hp致病机制的报道少见.为进一步探讨 Hp 在胃癌发生过程中的作用，我们对 Hp 根除前后胃癌前病变组织中 Bax 表达的变化进行了研究.

1 材料和方法

1.1 材料 我院 1994/1996 年胃镜活检标本 72 例.其中肠上皮化生 51 例，中、重度异型增生 21 例.胃镜活检标本均取自胃窦部.组织标本经 40 g/L 甲醛固定，石蜡包埋，4 μm 厚连续切片，并经 HE 染色病理诊断证实.Hp 检测采用快速尿素酶试验和 Warthy-starry 银染色，两项均阳性者为 Hp 阳性，两项均阴性者为 Hp 阴性，单项阳性者退出试验.对 Hp 阳性者给予有机铋剂、羟氨苄青霉素、灭滴灵三联药物进行治疗,疗程为 2 wk.治疗结束后复查胃镜，停药 4 wk 后 Hp 仍保持阴性者为 Hp 根除.

1.2 方法 Bax 蛋白表达的检测采用 SP 法.Bax 多克隆抗体为美国Dako公司产品，免疫组化试剂盒(Immunostain SP Kit)为美国 Santa Cruz 公司产品.染色程序按 SP 法操作常规进行.以 PBS 代替一抗为阴性对照，以已知 Bax 蛋白阳性的胃癌组织为阳性对照.光镜下观察 Bax 蛋白免疫组化染色切片的显色反应，数 5 个以上高倍视野，不少于 400 个胃黏膜上皮细胞.Bax 结合染色反应深度及阳性细胞数分为 0-3 四级:(-)无阳性反应细胞、阳性细胞<10 % 或背景同空白对照者；(+)10-25 % 细胞明显阳性,或大多数细胞弱阳性；(++)26-50 % 的细胞明显阳性；(+++)>50 % 的细胞明显阳性.

统计学处理 Stata 软件包的 χ^2 检验，Fisher 精确检验,有序的 Logistic 回归.P <0.05 有统计学意义.

2 结果

在 51 例肠化生组织中,Hp 阳性 35 例,Hp 阴性 16 例,Hp 感染率为 68.6 %.21 例中、重度异型增生组织中,Hp 阳性 12 例，Hp 阴性 9 例,Hp 感染率为 57.1 %.肠化生与异型增生组织中 Hp 感染率无显著性差异.

Bax 蛋白阳性反应物质呈棕黄色,主要位于腺上皮细胞质内,偶可伴胞核着色(图 1).Bax蛋白在肠化生和异型增生组织中均有不同程度的表达,其阳性表达率为 63.9 %.Hp 阳性组胃癌前病变 Bax 蛋白阳性表达率为 72.3 %,显著高于 Hp 阴性组(48.0 %,P <0.05).Hp 感染与 Bax 阳性表达及分级呈正相关(P <0.01,Logistic 回归分析,表 1).47 例 Hp 阳性患者经前述三联药物治疗后，37 例 Hp 被根除,10 例仍为 Hp 阳性.Hp 根除组胃癌前病变 Bax 蛋白阳性表达率为 43.2 %,较治疗前显著降低(70.3 %,P <0.05),而 Hp 仍为阳性组胃癌前病变 Bax蛋白阳性表达率(40.0 %)与治疗前无变化.

表 1 Hp 感染与胃癌前病变 Bax 蛋白表达的关系

Hp	*n*	Bax 阳性分级				Bax 阳性率(%)
		-	+	++	+++	
阳性	47	13	15	10	9	72.3[a]
阴性	25	13	6	4	2	48.0

[a]P <0.05, vs Hp 阴性组.

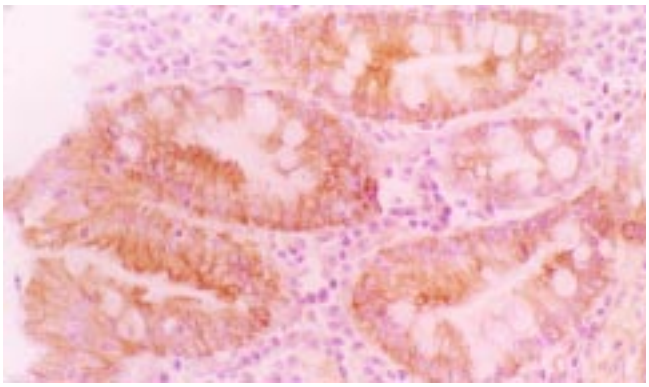

图 1 肠化生组织,Bax 蛋白阳性反应物质呈棕黄色 SP × 200.

3 讨论

胃癌发生发展过程中不仅存在细胞的过度增生,而且存在细胞凋亡的异常,细胞凋亡与细胞增生平衡失调是胃癌发生的病理学基础[19-28].Hp 是胃癌的第一类致病因子，其在胃癌发生中的作用已被广泛关注[1,2,8-14].我们以往的研究工作发现,Hp感染不仅可以刺激胃黏膜上皮细胞过度增生,也可以诱导胃黏膜上皮细胞凋亡,Hp 感染是引起胃黏膜上皮细胞增生和凋亡异常的重要机制之一;Hp感染后使胃黏膜上皮细胞增生和凋亡均明显增加，必然会导致胃黏膜上皮细胞增生与凋亡的调节紊乱，使胃黏膜上皮细胞不稳定性增加，从而增加患胃癌的危险性[20,23,24,28].但是，Hp 感染诱导胃黏膜上皮细胞凋亡及促进胃黏膜上皮细胞过度增生的机制目前尚不清楚.

Bax 是编码 Bcl-2 相关 X 蛋白(Bcl-2 associated X protein, Bax)的基因.Bax 蛋白与 Bcl-2 蛋白有 21 % 的同源性,具有对抗 Bcl-2 蛋白抑制细胞凋亡的作用.研究发现Bcl-2/Bax两蛋白之间的比例是决定对细胞凋亡抑制作用强弱的关键因素，因此认为Bax是重要的促细胞凋亡基因之一[29,30].Hp 感染是否通过影响 Bax 基因的表达调控胃黏膜上皮细胞增生和凋亡,是一个值得探讨的问题，因此，我们对胃癌前病变组织中 Hp 感染与 Bax 蛋白表达的关系进行了研究.研究发现,Bax 蛋白在肠化生和异型增生组织中均有不同程度的表达，Hp 阳性组胃癌前病变组织中 Bax 蛋白表达阳性率显著高于 Hp

阴性组,Hp感染与Bax阳性表达及分级呈正相关;Hp根除组胃癌前病变Bax蛋白阳性表达率较治疗前显著降低，而Hp仍为阳性组胃癌前病变Bax蛋白阳性表达率与治疗前无变化.表明Hp感染可以促进Bax蛋白的表达，这可能是Hp感染诱导胃黏膜上皮细胞凋亡的主要机制，导致胃黏膜上皮细胞增生与凋亡的调节紊乱，从而使胃黏膜上皮细胞易于向恶性转化.其原因可能是Hp感染继发炎症过程中释放多种细胞因子、自由基和一氧化氮，这些物质可引起染色体损伤、基因突变;也可能是Hp自身及其代谢产物有潜在的致癌活性[31-34].

本研究结果提示,对有Hp感染的胃癌前病变患者,应积极抗Hp治疗，可能会促使Bax基因异常表达的逆转，从而纠正细胞凋亡、细胞增生的异常，可能具有降低患胃癌的危险性，预防或减缓胃癌发生的重要作用.以上推论尚需临床观察验证.

4 参考文献

1 Vandenplas Y. *Helicobacter pylori* infection .*World J Gastroenterol* 2000;6:20-31
2 Liu HF, Liu WW, Fang DC, Wang GA,Teng XC.Relationship between *Helicobactor pylori* infection and gastric precancerous: a follow-up study. *Shijie Huaren Xiaohua Zazhi* 2002;10:912-915
3 Ji WS, Hu JL, Qiu JW, Peng DR, Shi BL, Zhou SJ, Wu KC, Fan DM. Polymor phism of flagellin A gene in *Helicobacter pylori*. *World J Gastroenterol* 2001;7:783-787
4 Xu KQ, Zhang WD, Wang JD, Li ZX, Zhou DY, Zhang YL, Huang WF,Jiang B, Sun Y. Cytotoxin of *Helicobacter pylori* promotes IL-8 secretion of gastric mucosa *in vitro*. *Shijie Huaren Xiaohua Zazhi* 2002;10:907-911
5 Peng XN, Fan XG, Huang Y, Wang ZM, Cheng YP. The study on relationship between helicobacter infection and primary liver carcinoma. *Shijie Huaren Xiaohua Zazhi* 2002;10:902-906
6 Gao HJ, Yu LZ, Bai JF, Peng YS, Sun G, Zhao HL, Miu K, Lü XZ, Zhang XY, Zhao ZQ. Multiple genetic alterations and behavior of cellular biology in gastric cancer and other gastric mucosal lesions: *H. pylori* infection, histological types and staging. *World J Gastroenterol* 2000;6:848-854
7 Miehlke S, Kirsch C, Dragosics B, Gschwantler M, Oberhuber G, Antos D, Dite P, Luter J, Labenz J, Leodolter A, Malfertheiner P, Neubauer A, Ehninger G, Stolte M, Bayerdrffer E. *Helicobacter pylori* and gastric cancer: current status of the Austrian-Czech-German gastric cancer prevention trial (PRISMA-Study). *World J Gastroenterol* 2001;7:243-247
8 Cai L, Yu SZ, Zhang ZF. *Helicobacter pylori* infection and risk of gastric cancer in Changle County, Fujian Province, China. *World J Gastroenterol* 2000;6:374-376
9 Xue FB, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of *H.pylori* infection with gastric carcinoma:a Meta analysis. *World J Gastroenterol* 2001;7:801-804
10 Yao YL, Xu B, Song YG, Zhang WD. Overexpression of cyclin E in Mongolian gerbil with *Helicobacter pylori*- induced gastric precancerosis. *World J Gastroenterol* 2002;8:60-63
11 Matsukura N. Relation between *Helicobacter pylori* and diseases: knowledge for clinician. *J Nippon Med Sch* 2002 ;69:200-2004
12 Miwa H, Go MF, Sato N. *H. pylori* and gastric cancer: the Asian enigma. *Am J Gastroenterol* 2002;97:1106-12
13 Wang GT. Progress in studies of mechanism of gastric precancerous lesions, carcinogenesis and their reversion. *Shijie Huaren Xiaohua Zazhi* 2000;8: 1-4
14 Zhuang XQ, Lin SR. Progress in research on the relationship between *Hp* and stomach cancer. *Shijie Huaren Xiaohua Zazhi* 2000;8:206-207
15 Yang Y, Deng CS, Yao XJ, Liu HY, Chen M. Effect of *Helicobacter pylori* on morphology and growth of gastric epithelial cells. *Shijie Huaren Xiaohua Zazhi* 2000;8: 500-504
16 Zhang ZW, Michael JG. Farthing. Molecular mechanisms of *H. pylori* associated gastric carcinogenesis. *World J Gastroenterol* 1999;5:369-374
17 Zhang XY. Some recent works on diagnosis and treatment of gastric cancer. *World J Gastroenterol* 1999;5:1-3
18 Han FC, Yan XJ, Su CZ. Expression of the CagA gene of *H. pylori* and application of its product. *World J Gastroenterol* 2000; 6:122-124
19 Jang TJ, Kim JR. Proliferation and apoptosis in gastric epithelial cells of patients infected with *Helicobacter pylori*. *J Gastroenterol* 2000; 35: 265-271
20 Liu HF, Liu WW, Fang DC, Yang SM, Wang RQ. *Bax* geng expression and its relationship with apoptosis in human gastric carcinoma and paracancerous tissues. *Shijie Huaren Xiaohua Zazhi* 2000;8:665-668
21 Zhang Z, Yuan Y, Gao H, Dong M, Wang L, Gong YH. Apoptosis, proliferation and p53 gene expression of *H. pylori* associated gastric epithelial lesions. *World J Gastroenterol* 2001; 7:779-782
22 Chan AO, Wong BC, Lam SK. Gastric cancer: past, present and future.*Can J Gastroenterol* 2001;15:469-474
23 Liu HF, Liu WW, Fang DC, Men RP. Expression and significance of proapoptotic gene *Bax* in gastric carcinoma. *World J Gastroenterol* 1999; 5: 15-17
24 Liu HF, Liu WW, Fang DC, Liu FX, He GY . Clinical significance of *Fas* antigen expression in gastric carcinoma. *World J Gastroenterol* 1999; 5: 90-91
25 Maeda S,Yoshida H, Hirata Y, Ogura K, Shiratori Y, Omata M. Analysis of apoptotic and antiapoptotic signalling pathways induced by *Helicobacter pylori*. *Gut* 2002;50:771-778
26 Wu YL, Sun B, Zhang XJ Wang SN, He HY, Qiao MM, Zhong J, Xu JY. Growth inhibition and apoptosis induction of sulindac on human gastric cancer cells. *World J Gastroenterol* 2001;7:796-800
27 Hamajima N, Matuo K, watanabe Y, Suzuki T, Nakamura T, Matsuura A, Yamao K, Tominaga S. A pilot study to evaluate stomach cancer risk reduction by *Helicobacter pylori* eradication. *Am J Gastroenterol* 2002;97:764-765
28 Liu HF, Liu WW, Fang DC, Gao JH,Wang ZH. Apoptosis and proliferation induced by *Helicobacter pylori* and its association with p53 protein expression in gastric epithelial cells. *Shijie Huaren Xiaohua Zazhi* 2001; 9:1265-1268
29 Maeda S, Yoshida H, Mitsuno Y, Hirata Y, Ogura K, Shiratori Y, Omata M. Analysis of apoptotic and antiapoptotic signalling pathways induced by *Helicobacter pylori*. *Gut* 2002;50:771-778
30 El-Rifai W, Powell SM. Molecular biology of gastric cancer. *Semin Radiat Oncol* 2002;12:128-140
31 Peek RM Jr, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas.*Nature Rev Cancer* 2002;2:28-37
32 Wang J, Chi DS, Kalin GB, Sosinski C, Miller LE,Burji I, Thomas E. *Helicobacter pylori* infection and oncogene expressions in gastric carcinoma and its precursor lesions.*Dig Dis Sci* 2002;47:107-113
33 Ebert MP, Leodolter A, Malfertheiner P. Novel strategies in the prevention of gastric cancer. *Hepatogastroenterology* 2001;48:1569-1571
34 El-Omar EM, Chow WH, Rabkin CS. Gastric cancer and *H. pylori*: Host genetics open the way.*Gastroenterology* 2001;121:1002-1004