• 研究快报 BRIEF REPORT •

不同胃黏膜疾病胃蛋白酶原 C 组织原位表达与胃蛋白酶原血清学检测水平的比较

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In situ expression and serum level of pepsinogen C in different gastric mucosa diseases

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Abstract

AIM: To explore the matching degree of *in situ* expression and serum level of pepsinogen C (PGC) in different gastric mucosal biopsies, and to evaluate its value in the diagnosis of gastric cancer.

METHODS: A total of 129 gastric mucosa biopsies and its corresponding serum specimens were collected from patients with superficial gastritis (n = 30), gastric ulcer or erosion (n = 35), atrophic gastritis (29), and gastric cancer (n = 35). The expression of PGC in the gastric mucosa was detected by immunohistochemistry, and the concentration of serum pepsinogen A (sPGA) and pepsinogen C (sPGC) were determined by enzyme linked immunosorbent assay (ELISA).

RESULTS: The positive rate of PGC antigen expression decreased in the tissues of superficial gastritis (100%), gastric ulcer or erosion (80.00%), atrophic gastritis (34.48%), and gastric cancer (11.43%) in sequence (P <0.05). The expression rate decreased as the increase of the disease severity. There was no statistical difference in the concentration of sPGA and sPGC among the above 4 groups. The ratio of sPGA to sPGC in the superficial gastritis, gastric ulcer or erosion, atrophic gastritis, and gastric cancer was 11.55±0.69, 9.39±0.86, 8.86±0.63, and 6.83±0.68, respectively (P <0.05), and decreased as the reduction of the PGC expression. There was specific correlation between the expression of PGC in gastric mucosa and the ratio of sPGA to sPGC in the serum (r = 0.297, P = 0.001).

CONCLUSION: Tissue expression of PGC has negative correlation with the severity of the gastric mucosal disease. The ratio of sPGA to sPGC is closely related with the tissue expression of PGC antigen, and it is a convenient and economic index for the screening and diagnosis of gastric cancer.

Key Words: Pepsinogen C; Pepsinogen A; Expression; Serology; Gastric cancer

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摘要

目的: 通过对不同类型胃黏膜活检组织胃蛋白酶原C(PGC)的表达和血清胃蛋白酶原含量的检测, 研究二者的匹配程度及其在胃癌筛查与诊断中的价值.

方法: 129例胃黏膜活检组织及其血清标本(浅表性胃炎30例, 胃黏膜糜烂溃疡35例, 萎缩性胃炎29例, 胃癌35例), 采用免疫组织化学染色检测胃黏膜标本中PGC的表达情况; 采用酶联免疫吸附实验检测血清PGA(sPGA)和PGC(sPGC)含量.

结果: 30例浅表性胃炎PGC抗原均为阳性表达, 表达率为100%, 而胃黏膜糜烂溃疡、萎缩性胃炎和胃癌组PGC抗原阳性表达率分别为80.00%, 34.48%和11.43%, 其表达率随疾病恶性程度的增加而下降(P<0.05). sPGC、sPGA含量各组间无统计学差异, sPGA/sPGC比值从浅表性胃炎→胃黏膜糜烂溃疡→萎缩性胃炎→胃癌依次下降, 分别为11.55±0.69, 9.39±0.86, 8.86±0.63, 6.83±0.68 (P<0.05). sPGA/sPGC比值随其病变组织PGC表达程度的降低而下降, 具有良好的相关性(r = 0.297, P = 0.001).

结论: PGC的组织表达与胃黏膜细胞恶性程度呈负相关. sPGA/sPGC与PGC抗原组织表达有良好的相关性, 是一个方

便、经济的胃癌及癌前病变筛选和诊断的指标, 具有较好的临床实用价值.

关键词: 胃蛋白酶原C; 胃蛋白酶原A; 表达; 血清学; 胃癌

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0 引言

胃癌普查和胃癌癌前疾病患者的定期随访是发现早期胃癌的重要手段[1,2], 简单、有效、经济、实用的普查方法是肿瘤学研究的热点, 多年来, 科研人员一直致力于探索诊断胃癌的生物学标志物的研究.

胃蛋白酶原(PG)是一种具有消化功能的内切蛋白酶, 属于天冬氨酸蛋白水解酶. 在酸性条件下被激活, 转化为具有消化功能的胃蛋白酶. 根据免疫学和生化学特点, 胃蛋白酶原可分为胃蛋白酶原A(PGA)和胃蛋白酶原C(PGC)[3]. 在人类, 胃蛋白酶原C最初出现于胚胎后期, 约胚胎的第32-36周, 是胃黏膜细胞分化成熟, 消化功能逐渐完善的一种标志[4]. 本实验室先期研究表明[5], PGC组织表达判定胃癌及其癌前疾病的灵敏度及特异度均高, 但胃黏膜组织标本只能通过胃镜活检或术后标本获得, 操作复杂, 成本较高, 作为胃癌普查手段受到一定限制, 因此我们进一步对这些患者的血清标本进行了胃蛋白酶原C的检测, 旨在探讨组织学PGC的表达水平与血清PGC浓度之间的关系, 同时检测血清PGA, 以探讨血清PG水平在胃癌诊断中的应用价值.

1 材料和方法

1.1 材料

1.1.1 实验对象 中国医科大学肿瘤研究所第三研究室收集的129例胃黏膜活检组织及其对应的血清标本, 包括浅表性胃炎30例, 胃黏膜糜烂溃疡35例, 萎缩性胃炎29例, 胃癌35例. 男76例, 女53例; 年龄16-80岁, 中位年龄51岁, 各病理组在性别及年龄构成上差异无统计学意义(P>0.05).

1.1.2 试剂 抗胃蛋白酶原C抗体(anti-pepsinogen C antibody, 商品名2D5)由日本临床检验研究所惠赠; 免疫组化用SP二步法试剂盒为福建迈新公司产品(Lot No: Kit-9801D2); PGC, PGA ELISA试剂盒购于芬兰百得公司(Cat. NO.6001080).

1.2 方法

1.2.1 胃蛋白酶原C的免疫组织化学染色(SP二步法) 实验过程按说明书操作. 结果判定根据细胞着色强度及阳性细胞数进行综合评分. 着色强度以多数细胞呈色程度为准, 凡细胞质或腺腔内黏液着浅棕色者为1分, 棕色者2分, 深棕色者为3分, 不着色为0分; 整块切片中阳性细胞占所有胃黏膜细胞中的比例<30%为1分, 30-70%为2分, >70%为3分, 无细胞染色为0分. 根据上述两项指标的积分数分为 4 级, 0分为阴性(-); 2-3分为(+); 4分为(++); 5-6分为(+++). ≥2分(+)为阳性表达; ≥4分(++～+++)为过表达或强阳性.

1.2.2 PGA、PGC血清ELISA检测 均严格按照试剂盒说明操作.

统计学处理 利用SPSS 10.0软件包进行统计学处理: PGC的免疫组织化学结果采用χ^2检验分析, PGA、PGC血清ELISA检测结果等计量资料采用多组间的单因素方差分析并作组间均数的两两比较, PGC组织原位表达与sPGA、sPGC浓度的关系应用Spearman等级相关分析, P<0.05有统计学意义.

2 结果

2.1 各类胃黏膜组织胃蛋白酶原C的动态表达 129例胃黏膜PGC免疫组织化学检测发现30例浅表性胃炎PGC抗原均为阳性表达, 表达率为100%, 而胃黏膜糜烂溃疡、萎缩性胃炎、胃癌PGC抗原阳性表达率分别为80.00%, 34.48%和11.43%. PGC抗原阳性表达率从浅表性胃炎→胃黏膜糜烂溃疡→萎缩性胃炎→胃癌依次逐渐下降. 由胃良性疾病到胃癌前疾病, PGC表达率显著下降(P<0.01); 由胃癌前疾病到胃癌, PGC表达率逐渐下降(P<0.05)(表1). PGC主要在细胞质及细胞膜着色, 细胞核内无阳性物质. 在浅表性胃炎和胃糜烂溃疡这类胃良性疾病中, PGC表达阳性细胞数多, 着色深. 在萎缩性胃炎和胃癌组织中PGC的表达呈灶状分布, 仅个别腺管或细胞中有表达, 并且阳性物质的着色较浅, 综合评分在3分以下.

表 1 不同胃黏膜病变PGC组织原位表达及血清PG浓度 (mean ± SD, μg/L)

	n	PGC阳性表达(%)	sPGA	sPGC	sPGA/sPGC
浅表性胃炎	30	100.00[b]	94.20 ± 7.79	10.20 ± 1.92	11.55 ± 0.69[b]
糜烂溃疡	35	80.00[bc]	92.51 ± 7.62	12.72 ± 1.84	9.39 ± 0.86
萎缩性胃炎	29	34.48[ad]	86.99 ± 7.64	12.22 ± 2.49	8.86 ± 0.63[c]
胃癌	35	11.43[d]	73.26 ± 6.09	14.80 ± 2.37	6.83 ± 0.68[d]

[a]P<0.05, [b]P<0.01 *vs* 胃癌组; [c]P<0.05, [d]P<0.01 *vs* 浅表性胃炎组.

2.2 不同胃疾病胃蛋白酶原含量血清学检测 129例患者进行胃黏膜组织学PGC检测的同时, 我们也检测了其血清PGA、PGC的水平. 本组测得sPGA含量从浅表性胃炎、胃黏膜糜烂溃疡、萎缩性胃炎到胃癌组依次降低, sPGC浓度从浅表性胃炎、胃黏膜糜烂溃疡、萎缩性胃炎到胃癌组有升高趋势, 但各组间比较均无统计学差异(P>0.05). 各组sPGA/sPGC比值从浅表性胃炎→胃黏膜糜烂溃疡→萎缩性胃炎→胃癌依次下降, 浅表性胃炎组与胃癌组比较有极显著统计学差异(P<0.01), 与萎缩性胃炎组相比有显著差异(P<0.05)(表1). 通过对本组PGC组织原位表达与sPGA、sPGC及sPGA/sPGC的Spearman等级相关分析发

现, sPGA/sPGC的比值下降与其病变组织原位PGC表达的降低呈伴随关系, 两者具有良好的相关性($r = 0.297$, $P = 0.001$).

3 讨论

PGC组织表达的动态变化可以体现不同胃黏膜的分化程度及胃疾病的恶性程度, 随着胃疾病加重, PGC表达减少. PGC表达产物的缺乏可能是胃黏膜细胞恶性转化的结果[6]. 胃底腺主细胞是胃蛋白酶原的主要来源, 胃癌常伴有胃黏膜萎缩, 导致主细胞被大量破坏, 胃黏膜分泌功能受损[7]; 同时由于恶性转化的细胞存在分化障碍, 致癌因子使胚细胞中的胃蛋白酶原基因受损突变, 导致胃黏膜细胞的终末分化产物PGC-Ag表达减少[8]. PGC作为胃分化成熟的一种功能蛋白酶, 在萎缩性胃炎表达显著下降, 说明这类胃疾病与胃良性疾病相比, 细胞分化障碍明显, 生物学特性更接近胃癌. 本研究结果表明, 随着疾病加重, 由胃良性疾病→胃癌前疾病→胃癌, PGC阳性表达率依次逐渐下降, 说明PGC的组织表达与胃癌的发生发展有良好的相关性.

胃蛋白酶原大部分分泌入胃腔, 一小部分酶原透过胃黏膜毛细血管入血. 血清胃蛋白酶原水平可反映胃蛋白酶分泌及胃黏膜的状态和功能[9-11]. 正常情况下, PGA为血液中PG的主要同功酶, 占总PG的75%, 血清PGA水平明显高于PGC. 这是由于主细胞合成与释放PGA入血速度较快, 代谢清除率较慢. 研究中通过对胃黏膜组织中PGC表达和血清PGC水平比较发现, 随着疾病加重, 胃黏膜组织中PGC表达率逐渐降低, 而其血清PGC水平有所升高, 两者呈不平衡势态, 但各组间sPGC无统计学差异. 究其原因可能由于PGC表达细胞分布较广, 胃外器官如乳腺及前列腺等也产生PGC[12-14], 因而局灶病变对其影响有限, 因此, 当细胞损伤时, 血清PGC的变化不明显, 表明单独应用血清PGC对胃癌的诊断无显著意义. 文献[15,16]报道, 胃癌患者血清PGA/PGC比值明显低于正常对照组人群, 对于胃癌的筛查及诊断有一定价值. 我们也发现, 随着患者胃疾病的加重, sPGA/sPGC比值呈逐步下降趋势, 胃良性疾病(浅表性胃炎)与癌前疾病(萎缩性胃炎)和恶性疾病(胃癌)相比差异显著, 表明胃黏膜疾病在从良性到恶性转化的这一质变过程中, 其细胞功能也发生了质的变化, 二者的比值对胃疾病的筛查和诊断具有实际应用价值.

我们通过对不同胃黏膜PGC表达程度及其血清sPGA/sPGC比值变化的比较发现, 随着胃黏膜疾病组织学恶性程度的增加, 组织PGC表达率下降, 同时血清PGA/PGC比值也下降, 二者呈伴随关系, 具有良好的相关性, 这一点互相验证了不同胃黏膜疾病PGC组织学表达程度及其血清PGA/PGC比值测定均可以作为胃癌及其癌前疾病筛查的可靠指标, 有学者曾将血清PGA/PGC比值誉为胃黏膜疾病的“血清学活检”[11,17].

总之, PGC作为胃疾病发生发展的判定指标在组织学上的意义更大, 而作为血清学的检查方法作用有限, 但如与血清PGA结合, 以sPGA/sPGC比值筛查胃黏膜疾病是一个较好的指标, 而且具有非侵入性、标本方便获得和成本较低的特点, 对胃黏膜疾病的大规模筛查具有实用价值.

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• 研究快报 BRIEF REPORT •

胃癌细胞 PTEN 甲基化与其表达的相关性

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Relationship between PTEN methylation and its mRNA expression in human gastric cancer cells *in vitro*

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Abstract

AIM: To investigate the relationship between the methylation status of the 5'CpG island of PTEN promoter region and the mRNA expression of PTEN gene in gastric cancer cell lines *in vitro*.

METHODS: The methylation status of PTEN promoter region and expression of PTEN mRNA in gastric cancer cell lines SGC-7901 (moderately differentiated), BGC-823 (lowly differentiated), MGC-803 (lowly differentiated), HGC-27 (non-differentiated) were measured by methylation-specific polymerase chain reaction (MSP) and semi-quantitative reverse transcription PCR, respectively.

RESULTS: The methylation of PTEN gene in promoter region was found in HGC-27, MGC-803, and BGC-823 cells, but not in SGC-7901 ones. HGC-27 cells had the highest methylation level of PTEN gene (138.217 ± 7.898, P <0.01), then MGC-803 and BGC-823 cells, and no significant difference was found between MGC and BGC (P >0.05). SGC-7901 cells had the highest expression of PTEN mRNA (0.336 ± 0.079, P <0.01), then BGC-823, MGC-803 and HGC-27 (lowest, 0.113 ± 0.047, P <0.05), and there was no significant difference between BGC and MGC (P >0.05). The expression of PTEN mRNA decreased gradually following the increase of the level of PTEN methylation. In addition,