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胰腺癌组织 ChAT，GAD65 和 PKC 酶活性的表达

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Enzymatic activities of ChAT, GAD65 and PKC in pancreatic carcinoma tissues

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Abstract

AIM: To study on the expression characteristics of ChAT, GAD65 and PKC enzymatic activities and their clinicopathological significance in the tissues of chronic pancreatitis and pancreatic carcinoma.

METHODS: The enzymatic activities of ChAT, GAD65 and PKC were detected by immunohistochemical method of avidin-biotin complex on formalin-fixed and routine paraffin-embedded sections of specimens of chronic pancreatitis (n =10) and pancreatic carcinoma (n =47).

RESULTS: The positive rate and the score of ChAT, GAD65 and PKC were significantly lower in 10 cases of chronic pancreatitis than that of pancreatic carcinoma (ChAT, 0 % *vs* 48.9 % , 0.2±0.4 *vs* 2.2±1.4; GAD65, 10.0 % *vs* 55.3 %, 0.6±0.9 *vs* 2.2±1.2; PKC, 10.0 % *vs* 57.4 %, 0.6±0.9 *vs* 2.1±1.6). The score of ChAT was significantly higher in well-differentiated adenocarcinoma than that of poorly-differentiated adenocarcinoma (P <0.05). The positive rate and the score of GAD65 or PKC were significantly lower (GAD65, P <0.05; PKC, P <0.01) in cases of well-differentiated adenocarcinoma than in cases of poorly-differentiated. No difference was found for the enzyme expressions and the clinicopathological characteristics among different sex, age, with or without metastasis of pancreatic carcinoma. A highly positive correlation was found between the scores of GAD65 and PKC in pancreatic carcinoma.

CONCLUSION: The expression of enzymatic activities of ChAT, GAD65 or PKC might be related to the carcinogenesis, progression and biological behaviors of pancreatic carcinoma. They might be important biological markers of pancreatic carcinoma.

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摘要

目的: 研究ChAT，GAD65和PKC 酶活性在慢性胰腺炎和胰腺癌组织中表达特征及其临床病理意义.

方法: 胰腺癌(n =47)和慢性胰腺炎(n =10)手术切除标本经40 g/L 中性甲醛固定后常规制作石蜡包埋切片，ChAT，GAD65和PKC 酶活性表达染色方法均为常规ABC免疫组化法.

结果: 胰腺癌 ChAT，GAD65 和 PKC 表达阳性率(48.9 %，55.3 % 和 57.4 %)及其评分(2.2 ± 1.4，2.2 ± 1.2 和 2.1 ± 1.6)明显高于慢性胰腺炎阳性率(0 %，10.0 % 和 10.0 %)及其评分(0.2 ± 0.4，0.6 ± 0.9 和 0.6 ± 0.9)，均有显著或高度显著性差异(P <0.05 或 P <0.01). 高分化腺癌ChAT评分值明显高于低分化腺癌(P <0.05)，但阳性率之间无明显差异(P >0.05);高分化腺癌 GAD65，PKC 表达阳性率及其评分明显低于低分化腺癌，均有显著或高度显著性差异(P <0.05 或 P <0.01). 酶活性表达与胰腺癌患者性别、年龄、有无转移等临床特征均无明显关系. GAD65 评分与 PKC 评分存在高度密切关系(r =0.50，P <0.01)

结论: ChAT，GAD65 和 PKC 酶活性表达特征可能与胰腺癌发生发展及生物学行为有密切关系，均为胰腺癌 重要生物学标志物.

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0 引言

胆碱乙酰转移酶(choline acetyltransferase，ChAT)在胆碱能神经元和神经纤维及细胞运动中起重要调节作用，是胆碱能神经元特异性标志物[1-6]. 谷氨酸脱羧酶(glutamate decarboxylase，GAD)有两种异构体(即 GAD65 和 GAD67)，二者与 γ - 氨基丁酸(GABA)能系统的功能代谢存在密切关系，是 GABA 合成的关键酶[7-9]. 蛋白激酶 C (protein kinase C，PKC) 在信号转换、细胞分化和生长过程中起重要作用[10-13]. 新近发现ChAT表达活性与恶性肿瘤细胞发生及侵袭潜能有关[14-16]，GAD65 和 PKC 表达活性

与恶性肿瘤发生发展及生物学行为有较密切关系[17-30]. 国内外尚未见胰腺癌该方面研究的文献报道. 我们应用免疫组化方法研究胰腺癌和慢性胰腺炎组织中 ChAT, GAD65 和 PKC 酶活性表达及其生物学意义.

1 材料和方法

1.1 材料 收集湘雅医院和湘雅二医院1996-01/2001-12手术切除胰腺癌标本 47 例 (术前均未进行化疗和放疗), 男 35 例. 女 12 例, 年龄 21-73 (平均 50 ± 12)岁. 均来源于胰腺导管上皮, 包括高分化腺癌 19 例, 中分化腺癌 8 例, 低分化腺癌 17 例和黏液腺癌 3 例. 临床和(或)病理证实发生胰腺外转移(区域淋巴结或胰腺外组织器官)36 例 (76.6 %). 另收集慢性胰腺炎手术切除标本 10 例, 男 6 例, 女 4 例, 年龄 35-65(平均 42 ± 7 岁). 上述标本经 40 g/L 中性甲醛固定后, 常规制作石蜡包埋连续切片, 切片厚 4 μm. 兔抗人 ChAT, GAD65 多克隆抗体, 鼠抗人 PKC 单克隆抗体, 生物素标记羊抗兔 IgG, 生物素标记羊抗鼠 IgG, ABC 试剂, 以及 DAB-HCL 显色试剂盒均购自武汉博士德公司.

1.2 方法 ChAT, GAD65 和 PKC 表达染色方法均为常规 ABC 免疫组化法(具体染色步骤略), 镜下观察 5 个高倍镜视野染色结果, 细胞质内含棕黄色颗粒者为 ChAT 和 GAD65 阳性细胞, 细胞膜含棕黄色颗粒者为 PKC 阳性细胞; 根据免疫组化着色强度评分(0 分, 无; 1 分, 弱; 2 分, 中; 3 分, 强)和阳性细胞率(0 分, 小于 5 %; 1 分, 5-10 %; 2 分, 11-20 %; 3 分, 21-50 %; 4 分, 大于 50 %)之和为该病例评分值, 且将评分值 0-2 分定为阴性表达病例(-), 大于 2 分定为阳性表达病例(+). 以 0.05 mol/L PBS 液(PH7.4)代替一抗作为染色阴性对照或替代对照, 以多次替代对照有效的乳腺癌切片作为染色阳性对照.

统计学处理 将所得数据输入 SPSS10.0 软件包进行 χ^2 检验, t 检验及 Fisher's 精确概率法, 检验水准 α =0.05 和 α =0.01.

2 结果

ChAT 和 GAD65 免疫组化反应产物主要定位于细胞质, 偶见细胞核着色(图 1, 2). PKC 免疫组化反应产物主要定位于细胞膜, 部分病例定位于细胞膜和细胞质(图 3). 三者在癌组织中分布呈较明显异质性, 同一切片中癌组织内阳性细胞着色强度和阳性细胞率可有较明显不同. 慢性胰腺炎导管上皮三者阳性率及其评分明显低于胰腺癌, 均有显著或高度显著性差异(P <0.05 或 P <0.01, 表 1). 高分化腺癌 ChAT 表达阳性率及其评分明显高于中、低分化腺癌, 与低分化腺癌相比其评分有显著性差异(P <0.05). 低分化腺癌 GAD65、PKC 表达阳性率及其评分明显高于中、高分化腺癌, 与高分化腺癌比较均有显著或高度显著性差异(P <0.05 或 P <0.01). 三者表达阳性率与性别、年龄及有无转移均无明显关系(P >0.05, 表 2). ChAT 表达评分与 GAD65、PKC 表达评分无明显相关性(P >0.05), 但 GAD65 与 PKC 表达评分呈密切正相关(r =0.50, P <0.01).

表 1 胰腺癌组织 ChAT, GAD65 和 PKC 酶活性表达

分组	n	阳性率(%)			评分值($\bar{x} \pm s$)		
		ChAT	GAD65	PKC	ChAT	GAD65	PKC
胰腺炎	10	0.0	10.0	10.0	0.2 ± 0.4	0.6 ± 0.9	0.6 ± 0.9
胰腺癌	47	48.9[b]	55.3[a]	57.4[a]	2.2 ± 1.4[b]	2.2 ± 1.2[b]	2.1 ± 1.6[a]

[a]P <0.05, [b]P <0.01 *vs* 胰腺炎.

表 2 胰腺癌临床病理特征与 ChAT, GAD65 和 PKC 表达的关系

	n	阳性率(%)			评分值($\bar{x} \pm s$)		
		ChAT	GAD65	PKC	ChAT	GAD65	PKC
男	35	48.6	51.4	57.1	2.3 ± 1.5	2.1 ± 1.3	2.1 ± 1.5
女	12	50.0	66.7	58.3	2.0 ± 1.1	2.6 ± 0.9	2.1 ± 1.7
年龄小于 45	15	40.0	60.0	66.7	2.2 ± 0.9	2.5 ± 1.0	2.4 ± 1.5
大于或等于 45	32	53.1	53.1	53.1	2.2 ± 1.6	2.1 ± 1.3	2.0 ± 1.6
高分化腺癌	19	63.1	42.1	47.3	2.7 ± 1.4	1.8 ± 1.4	2.0 ± 1.5
中分化腺癌	8	37.5	50.0	37.5	1.9 ± 1.5	2.2 ± 0.9[b]	1.2 ± 1.5
低分化腺癌	17	35.3	76.5[a]	82.4[a]	1.8 ± 1.2[a]	2.8 ± 0.9[b]	2.8 ± 1.5[c]
黏液腺癌	3	66.7	33.3	33.3	2.0 ± 1.7	1.3 ± 1.5	1.3 ± 1.5[d]
转移 无	11	45.5	54.5	63.6	2.5 ± 1.6	2.1 ± 1.6	2.1 ± 1.6
有	36	50.0	55.6	55.6	2.1 ± 1.3	2.3 ± 1.1	2.1 ± 1.6

[a]P <0.05, [b]P <0.01 *vs* 高分化腺癌, [c]P <0.01, [d]P <0.01 *vs* 低分化腺癌.

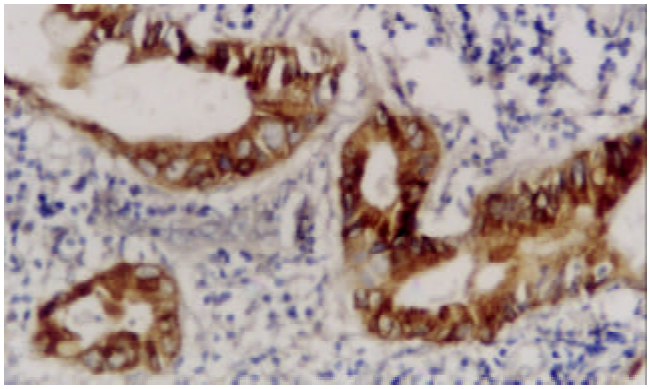

图1 胰腺高分化腺癌ChAT酶活性阳性表达ABC法×100.

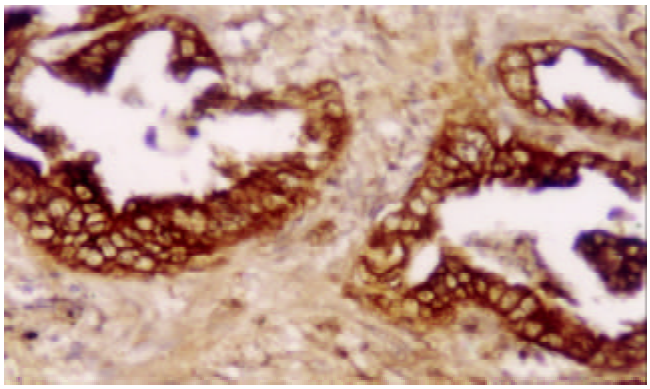

图2 胰腺中分化腺癌GAD65酶活性阳性表达ABC法×100.

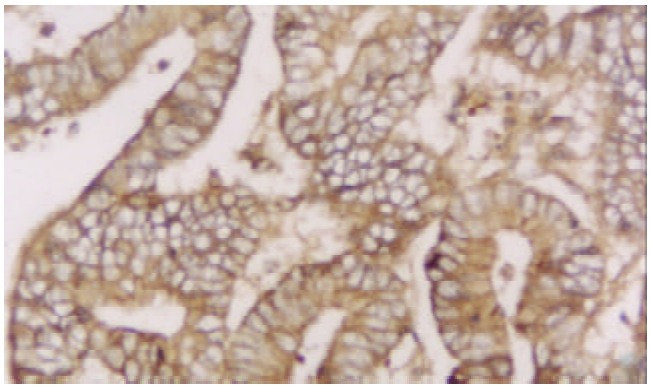

图3 胰腺高分化腺癌PKC酶活性阳性表达ABC法×100.

3 讨论

ChAT可能与肿瘤发生发展及生物学行为有密切关系，人类原发和转移性黑色素瘤存在ChAT和蝇覃碱乙酰胆碱受体的表达，而正常皮肤黑色素细胞则缺乏，人类黑色素瘤细胞株(SK-mel28)中，ChAT通过其受体调节瘤细胞收缩和运动，认为与瘤细胞侵袭潜能有密切关系. 人类小细胞肺癌、畸胎瘤和神经母细胞瘤中ChAT能促进癌细胞生长和增强其侵袭和发生转移的能力[14-16]. ChAT在其他恶性肿瘤中是否表达尚未文献报道. 我们发现慢性胰腺炎导管上皮均呈ChAT阴性表达，胰腺癌阳性表达率48.9%，且高分化腺癌ChAT评分明显高于低分化腺癌，但与胰腺癌是否转移无明显关系. 提示部分胰腺癌表达ChAT，说明部分上皮性恶性肿瘤象黑色素瘤一样存在ChAT，ChAT可能通过其受体作用调节肿瘤细胞生长、分化、运动及其生物学行为，其确切作用机制有待深入研究.

GAD65为γ-氨基丁酸(GABA)合成的关键酶，而GABA源性系统在调节细胞生长发育等方面起重要作用，应用GABA受体拮抗剂处理后可抑制多种细胞的生长发育[7-9]. 近年研究发现恶性肿瘤(如结肠癌、乳腺癌、胃癌等) GAD65表达水平明显高于其正常组织和良性病变，认为GAD65表达与恶性肿瘤发生、生物学行为等有密切关系[17-20]. 我们发现慢性胰腺炎导管上皮GAD65表达阳性率及其评分明显低于胰腺癌，高分化腺癌阳性率及其评分明显低于低分化腺癌，但与是否转移无明显关系. 其结果与国外文献报道较一致，说明GAD65表达特征与胰腺癌发生发展及生物学行为存在密切关系，为胰腺癌重要生物学标志物之一.

PKC是一大类结构功能不同的丝氨酸/苏氨酸激酶家族，至少有12种同工酶亚型. PKC广泛分布于哺乳动物组织细胞中，具有多种生理功能，是一系列细胞级联信号途径的关键环节[10-13]. 近年研究发现PKC表达在恶性肿瘤生物学行为及肿瘤细胞多药耐药性形成方面有重要作用，恶性肿瘤PKC表达水平明显高于正常组织及其良性病变，多药耐药胃癌细胞株PKC表达水平明显高于药物敏感细胞株[21-30]. 本结果显示慢性胰腺炎中导管上皮PKC表达阳性率及其评分明显低于低分化腺癌，但与有无转移无明显关系. 说明PKC表达与胰腺癌发生及其恶性程度有密切关系，检测PKC表达水平对指导临床胰腺癌化疗或放疗可能具有重要临床应用价值. 其机制可能与PKC本身生物学作用有关.

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