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肝癌组织 HSP70 和 caspase 3 的表达意义

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Expression of HSP 70 and caspase 3 and their significance in hepatocellular carcinoma tissues

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Abstract

AIM: To investigate the expression of heat shock protein 70 (HSP70) and caspase 3 protein and their clinical significance in hepatocellular carcinomas and surrounding liver tissues.

METHODS: The expression of HSP70 and caspase 3 protein were detected by immunohistochemistry in hepatocellular carcinomas (HCC) and their surrounding liver tissues.

RESULTS: The positive rate and intensity of HSP70 in HCCs were significantly higher than those in pericarcinomatous liver tissues (68.6% *vs* 31.4%, P <0.01), and these of caspase protein were significantly lower (17.1% *vs* 35.7%, P <0.01). The expression level of HSP70 and caspase protein in HCCs was remarkably related to differentiation degree and tumor size of HCCs, and the poorer differentiation, the stronger the expression of HSP70 (F =5.219 and 5.421 respectively, P <0.01), the weaker the expression of caspase 3 protein (F =5.944 and 4.571 respectively, P <0.01). The correlation analysis indicated that there was a negative relationship between expression of HSP70 and caspase protein in HCC and their surrounding liver tissues (r = 0.4 126 and 0.5 237 respectively, P <0.01).

CONCLUSION: The expression of HSP70 may make uncontrolled growth and unceasingly increased malignant degree of HCC by accelerating cell transformation and proliferation and inhibiting apoptosis. HSP70 may be an important marker for evaluation of prognosis in patients with HCC.

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摘要

目的: 研究热休克蛋白70和caspase 3在肝细胞癌及癌旁肝组织中的表达及其临床意义.

方法: 利用免疫组织化学检测70例肝细胞癌及其癌旁肝组织中 HSP70 和 caspase 3 蛋白的表达.

结果: HCC中HSP70的阳性率和阳性强度明显高于癌旁肝组织(68.6% *vs* 31.4%, P <0.01), 而 caspase3 蛋白的阳性率和阳性强度明显低于癌旁肝组织(17.1% *vs* 35.7%, P <0.01). 在HCC中HSP70和caspase3蛋白的表达强度与HCC的分化程度和肿瘤的大小明显相关, 分化愈差和肿瘤愈大HSP70表达愈强(分别为: F =5.219和5.421, P均<0.01)而caspase3蛋白表达愈弱(分别为 F=5.944和4.571, P均 <0.01). 统计学分析显示在 HCC 及其癌旁肝组织中 HSP70 和 caspase 3蛋白的表达呈明显负相关(分别为: r =-0.4 126和-0.5237, P均<0.01).

结论: 本文结果提示在HCC发生过程中HSP70的表达可能通过促进细胞的转化和增生及抑制细胞的凋亡, 使HCC呈现无限制的生长及恶性程度不断增高; 检测HSP70有望能成为判断HCC预后的重要指标之一.

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0 引言

在我国及日本肝细胞癌(HCC)是一种死亡率非常高的高度恶性肿瘤[1-10], 其发生与肝炎病毒感染密切相关[11-14], 并呈多步骤多阶段的特性[15-16]. 尽管HCC的研究和治疗在近年取得了很大的进展[17-26], 但其发生的分子机制及比较特异的诊断标记物和治疗靶分子均未得到明确的鉴定. 最近Chuma *et al*[27]利用基因芯片技术研究发现热休克蛋白 70(HSP70)可能是一种鉴别早期 HCC 的有效分子标记物. HSP70是一组重要应激蛋白. 在肿瘤细胞中表达异常, 能与癌基因、抑癌基因产生结合, 并与肿瘤细胞的细胞周期调控、增生、凋亡、分化、多药耐药、肿瘤免疫以及肿瘤细胞的发生发展密切相关[28], caspase 3 是凋亡信号转导通路中的重要执行分子, 其激活后导致细胞的不可逆性凋亡[29]. 我们利用组织芯片技术和免疫组织化学方法检测 HSP70 和 caspase 3 在 HCC 及其癌旁肝组织中的表达, 并探讨二者的相关性及其临床意义.

1 材料和方法

1.1 材料 1996-01/2003-02湘雅医院肝胆外科手术切除的 HCC 标本 70 例(均附癌旁肝组织)及胆囊炎患者切除的正常肝组织 10 例. 所有标本经 40 g/L 甲醛固定，常规石蜡包埋、切片、病理学确诊. 在 70 例 HCC 中男 53 例，女 17 例，平均年龄 53.4 (27-68 岁); 肿瘤直径小于或等于 5 cm 者 25 例; 5.1-10 cm 者 29 例; 大于 10 cm 者 16 例. 其中肝内有多个肿瘤结节者 19 例; 患者术前均未做过任何放疗和化疗. HCC 组织根据 Edmondson 标准分级，其中Ⅰ级 11 例，Ⅱ级 31 例，Ⅲ级 23 例，Ⅳ级 5 例. 所有癌旁肝组织均有不同程度的肝纤维化和肝硬化，其中有非典型增生者 22 例. 抗 HSP70、抗磷酸化 Caspase3蛋白抗体及二步法Elivision试剂盒均购自福州迈新生物技术开发公司.

1.2 方法 利用自行设计制作的模具制备含 96 孔直径 2 mm 的蜡胚; 在显微镜下 HE 切片，选取要检测的区域，在石蜡包埋块上用孔径 1.8 mm 的骨髓穿刺针穿下对应区域的组织，灌入制备好的蜡胚的小孔中，并使各组织柱表面平整，在切片机上切取 4 μm 的连续切片，60 ℃烤片备用(制作组织芯片). 切片脱蜡至水; 30 mL/L H_2O_2阻断内源性过氧化物酶30 min; 高火档微波修抗原 5 min; 然后按免疫组织化学二步法操作. 用 PBS 代替一抗作阴性对照. 参照Bresalier半定量公式判断染色结果: 在每张切片中随机选取 10 个视野，根据细胞染色强度分为 4 级，并分别计分: 阴性，细胞无着色(0); 弱阳性，细胞着色为浅黄色(1); 中度阳性，细胞着色为棕黄色(2); 强阳性，细胞着色为棕褐色(3). 计数每一强度的视野数，根据下列计算公式计算每张切片的平均染色强度: IS(intensity score)= ∑{(0 × F0)+(1 × F1)+(2 × F2)+(3 × F3)}，*F* =% × 10 视野.

统计学处理 用SPSS10.0统计软件进行χ^2检验及相关性分析.

2 结果

2.1 HSP70 和 caspase3 蛋白的表达 HSP70 和 caspase3 蛋白的阳性信号主要位于细胞质，少数细胞核阳性(图 1，2)，在 HCC 及癌旁肝组织中 HSP70 蛋白的阳性率分别为68.6%和31.4%，正常肝组织10例中仅2例呈弱阳性，HCC 中的阳性表达率明显高于癌旁肝组织(P <0.01). caspase3 蛋白在 HCC 和癌旁肝组织中的阳性率分别为17.1%和35.7%，正常肝组织的阳性率为80%，HCC中的阳性表达率明显低于癌旁肝组织和正常肝组织(P<0.01).

2.2 HSP70 和 caspase3 表达与 HCC 在 HCC 中 HSP70 和 caspase3蛋白的表达强度与HCC的分化程度明显相关，分化愈差 HSP70 表达愈强，而 caspase3 蛋白表达愈弱，反之亦然(P<0.01，表 1); HCC 肿块愈大 HSP70 蛋白表达强，而caspase3蛋白表达愈弱，反之亦然(P<0.01，表1).在HCC和癌旁肝组织中HSP70和caspase3蛋白的表达强度呈明显负相关(分别为 r =-0.4 126 和 -0.5 237，P <0.01，表 1).

表 1 HSP70 和 caspase3 表达与 HCC 病理关系

HCC	*n*	HSP70 蛋白表达(IS)	caspase3 蛋白表达(IS)
分级			
Ⅰ	11	0.78 ± 0.43	1.49 ± 0.65
Ⅱ	31	1.64 ± 1.05[a]	1.52 ± 0.95[b]
Ⅲ	23	1.81 ± 1.10[a]	0.80 ± 0.57[b]
Ⅳ	5	2.01 ± 0.84[a]	0.46 ± 0.24[b]
肿块直径			
≤ 5 cm	25	1.11 ± 0.88	1.42 ± 0.87
5.1-10 cm	29	1.88 ± 1.07[c]	1.01 ± 0.99[d]
> 10 cm	16	2.02 ± 0.77[c]	0.63 ± 0.44[d]
总计	70	1.59 ± 1.21	1.07 ± 0.98
癌旁肝组织	70	1.07 ± 0.98	1.87 ± 1.10

[a]P <0.01, [b]P <0.01 *vs* Ⅰ级, [c]P <0.01, [d]P <0.01 *vs* ≤ 5 cm.

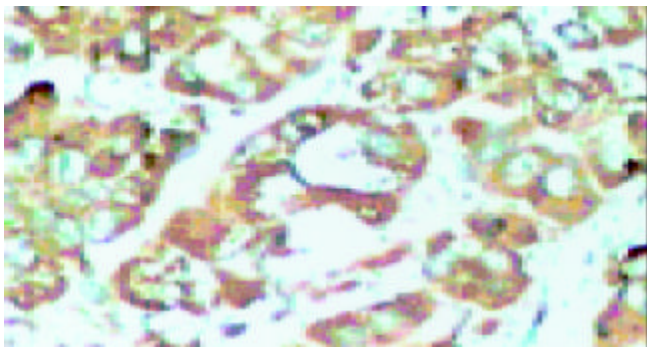

图 1 HCC 胞质中 HSP70 阳性. SP×400.

图 2 HCC 胞质中 Caspase 阳性, 部分胞核阳性. SP×400.

3 讨论

既往报道在多种肿瘤和转化的培养细胞中 HSP70 呈过表达，对维持肿瘤细胞的增生非常重要[30-32]. 本结果显示 HSP70 在 HCC 中的表达率和表达强度均显著高于癌旁肝组织，并与 HCC 的分化程度和肿瘤的大小明显相关，分化愈差表达愈强，肿瘤愈大表达愈强，与尹燕明 *et al* 的结果基本一致. Chuma *et al*[27]利用基因芯片技术研究热休克蛋白 70 在 HCC 中的表达，并认为检测 HSP70 蛋白的表达可作为早期肝细胞癌的重要诊断指标. 根据我们的资料 HSP70 有望能成为判断 HCC 预后的重要指标之一.

在HCC的发生中常存在细胞增生和细胞凋亡的失衡，caspase 3是细胞凋亡通路中重要的执行分子，其以磷酸化方式激活后导致细胞发生不可逆性凋亡[29, 33]，利用免疫组织化学检测磷酸化caspase 3蛋白的表达，能基本反映细胞凋亡的状况. 本资料显示HCC中磷酸化caspase3蛋白的表达率和表达强度均显著低于癌旁肝组织，亦与HCC的分化程度和肿瘤大小明显相关，分化愈差和瘤块愈大其表达愈弱. 提示在HCC的发生发展过程中可能存在癌细胞的低凋亡.

HSP70是否具有直接调节细胞凋亡的作用，目前尚无定论，尹燕明 *et al* 研究结果显示HCC中HSP70的表达与细胞凋亡指数呈明显正相关; Chen *et al* 发现表达HSP70的HepG2细胞能显著抑制UVC诱导的凋亡; 赵霞 *et al* 利用反义寡核苷酸阻断HSP70的表达可诱导卵巢癌细胞的凋亡; 本文结果显示在HCC和癌旁肝组织中HSP70和caspase3蛋白的表达强度呈明显负相关. 推测在HCC发生过程中HSP70的表达可能通过促进细胞的转化和增生及抑制细胞的凋亡，使HCC呈现无限制的生长及恶性程度不断增高.

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