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大肠腺癌组织 Survivin 蛋白的表达意义

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Expression of survivin protein in colorectal adenocarcinoma

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Abstract

AIM:To investigate the expression of survivin and its relationship with proliferation and apoptosis in colorectal adenoma and adenocarcinoma.

METHODS:Using terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and immuno histochemistry S-P method, the authors examined the expression of survivin, Ki-67 and apoptotic cell in situ in 60 cases of colorectal adenocarcinoma, 35 adenoma and 20 normal colonic mucosa.

RESULTS:Survivin expression was observed in 36 of 60 (60.0 %) cases of colorectal adenocarcinoma and in 6 of 35 (17.1 %) cases of adenoma. In contrast, normal colonic mucosa did not express survivin. Overexpression of survivin was related to the differentiation grade of colorectal adenocarcinoma, however there was no correlation with Dukes's stage of lymph node metastasis. Ki-67 labeling index (LI) was higher in colorectal adenocarcinoma than that in adenoma (39.1± 10.4 % versus 22.3 ± 6.2 %, P <0.01). The apoptosis index (AI) of colorectal adenocarcinoma and adenoma was significantly higher than that of normal tissues (P <0.01). More apoptotic cells were noticed in well and moderate differentiated adenocarcinoma than those in poorly differentiated adenocarcinoma (P <0.05). Survivin positive adenoma and adenocarcinoma had significantly lower values for AI than survivin negative tumors (P <0.01), and the Ki-67 LI in survivin positive adenoma and adenocarcinoma were higher than that in survivin negative tumors (P <0.01).

CONCLUSION:Up-regulation of survivin expression in adenocarcinoma suggests that survivin may play an important role in human colorectal tumorigenesis through the inhibition of apoptosis and acceleration of proliferative activity. Survivin may be a new prognostic implication in colorectal adenocarcinoma and serve as a widely applicable target for anticancer gene therapy.

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摘要

目的:检测凋亡抑制蛋白 Survivin 在大肠腺瘤、腺癌组织中的表达，观察其与细胞增生、凋亡的关系，探讨 Survivin 基因表达在大肠癌发生、发展过程中的作用.

方法:应用 DNA 缺口末端标记技术和免疫组织化学 S-P 法，原位观察 20 例正常大肠黏膜组织、35 例大肠腺瘤和 60 例大肠腺癌组织中的凋亡细胞和 Survivin 、Ki-67 蛋白阳性表达.

结果:Survivin 蛋白在正常大肠黏膜组织中不表达，在大肠腺癌和腺瘤中阳性表达率分别为 60.0 % 和 17.1 %，二者比较差异有显著性(P <0.01). Survivin 蛋白表达与大肠腺癌分化程度呈负相关，与 Dukes ' 分期和淋巴结转移无明显相关. 大肠腺癌 Ki-67 标记指数(labeling index，LI)为 39.1 ± 10.4 %，较腺瘤 22.3 ± 6.2 % 显著增高(P <0.01)，大肠腺癌Survivin阳性组Ki-67 LI 25.1±7.6 %与Survivin阴性组 19.7 ± 5.8 % 比较差异有显著性(P <0.01). 大肠腺瘤和腺癌细胞凋亡指数(AI)显著高于正常大肠黏膜(P <0.01)，高分化癌凋亡指数显著高于低分化癌(P <0.05)，大肠腺癌 Survivin 阳性组凋亡指数 0.85 ± 0.52 % 与 Survivin 阴性组 1.25 ± 0.58 % 比较差异有显著性(P <0.01).

结论:Survivin 基因在大肠腺癌组织中表达上凋，通过促进肿瘤细胞增生和抑制凋亡参与大肠癌的发生、发展过程. 检测大肠组织 Survivin 蛋白表达，对预测癌变及判断预后有重要意义. Survivin基因为肿瘤的基因治疗提供一理想靶点.

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0 引言

大肠癌是世界上高发恶性肿瘤之一，在我国为 4-6 位[1,2]. 细胞凋亡调节紊乱是大肠癌的重要机制之一[3-13]. Survivin 是新近发现的凋亡抑制蛋白(inhibition apoptosis protein，IAP)，在正常成熟组织中不表达，在人类各

种肿瘤组织广泛表达[14-20]，其独特的结构和生物学功能在肿瘤分子生物学研究中日益受到重视. 本研究通过检测Survivin蛋白在大肠腺瘤和腺癌中的表达，观察其与细胞增生、凋亡的关系，探讨 Survivn 基因表达在大肠癌发生、发展过程中的作用.

1 材料和方法

1.1 材料 大肠腺癌 60 例、大肠腺瘤 35 例和正常大肠黏膜 20 例均取自武汉大学中南医院、湖北省肿瘤医院 1999-2001 年经病理证实的手术切除标本和活检组织，40 g/L 甲醛固定，石蜡包埋 4 μm 连续切片，HE 染色复诊. 所有标本均选自术前未经放、化疗且无坏死的组织. 60例大肠腺癌中，按癌组织分化程度分为高分化11例，中分化 23 例，低分化 26 例，男 34 例，女 26 例，年龄 26-75 岁，平均 52.6 岁. Dukes ’ A 期 10 例，B 期 32 例，C 期 18 例. 35 例大肠腺瘤中，男 17 例，女 13 例，年龄 21-70 岁，平均 49.5 岁，其中管状腺瘤 21 例，绒毛状腺瘤 6 例，管状绒毛状腺瘤 8 例.按不典型增生程度分为轻度不典型增生13例，中度不典型增生16例，重度不典型增生6例. Survivin 兔抗人多克隆抗体(RB-1629)为美国 Neomarkers 公司产品(购自晶美公司)，Ki-67 鼠抗人单克隆抗体(即用型)、S-P 试剂盒均购自福州迈新公司，TUNEL 试剂盒购自德国宝灵曼公司.

1.2 方法 组织切片常规脱蜡至水，微波抗原修复，S-P 法操作，DAB 显色，苏木精复染. Survivin 工作浓度为 1∶1 000. 将已知的大肠癌切片作阳性对照，用磷酸盐缓冲溶液(PBS)代替一抗作阴性对照. 采用 DNA 末端标记方法检测组织中凋亡的细胞，染色方法按原位凋亡细胞检测试剂盒说明书进行，以已知大肠癌阳性片作阳性对照，以PBS代替 TDT 工作液作阴性对照. 凋亡细胞的判断根据细胞核呈棕黄色染色并在形态学上符合凋亡细胞特征，在高倍镜(×400)下计数 1 000 个细胞中的阳性细胞，以百分数表示，计数结果作为凋亡指数(apoptosis index ，AI). Ki-67 抗原以细胞核出现浅至深棕黄色颗粒作为阳性，在高倍镜(× 400)视野中计数 1 000 个细胞中阳性细胞的百分比作为 Ki-67 标记指数(Ki-67 labeling index). Survivin 阳性判定参考 Kawasaki et al [21]报道的方法：计数 5 个高倍视野，将平均阳性细胞数分为 5 类. 0，<5 %；1，5-25 %；2，25-50 %；3，50-75 %；4，>75 %. 根据染色程度将阳性信号分为3类. 染色强度弱，1+；中等染色强度，2+;染色强度强，3+. 染色强度×阳性细胞百分数为每个病例染色的综合记分，综合计分<1 为表达阴性，反之判定为阳性.

统计学处理 所取数据以 $\bar{x} \pm s$ 表示，用方差分析或 χ^2 检验，以 P <0.05 为差异有显著性.

2 结果

2.1 Survivin，Ki-67 表达和细胞凋亡 Survivin 蛋白阳性反应物主要位于细胞质中，为棕黄色颗粒(图 1)，正常大肠黏膜组织中未见Survivin蛋白表达(图2). 大肠腺癌、腺瘤中Survivin蛋白阳性表达率分别为60.0 %(36/60)，17.1 %(6/35)，二者比较差异有显著性(P <0.01). Ki-67 阳性反应产物主要位于细胞核内，呈浅至深黄色颗粒(图 3)，正常大肠黏膜基底层细胞有 Ki-67 的表达，大肠腺癌 Ki-67 标记指数 39.1 ± 10.4 %，显著高于腺瘤 22.3 ± 6.2 %，具有较高的增生活性(P <0.05). 凋亡阳性细胞在组织中为单个散在或小簇状分布，核物质呈棕黄色，大肠正常黏膜凋亡阳性细胞数量很少，多位于表层上皮中，腺瘤和腺癌组织检测到较多的凋亡的细胞(图 4)，大肠腺癌、腺瘤凋亡指数分别为 0.75 ± 0.44 %、1.64 ± 1.15 %，二者比较差异有显著性(P <0.01).

2.2 Survivin 表达与大肠腺癌临床病理的关系 Survivin 蛋白表达与大肠腺癌组织分化程度相关，低分化组的Survivin蛋白阳性表达率(19/26，73.1 %)显著高于中分化组(12/23，52.2 %)和高分化组(5/11，45.5 %，P <0.05)，而与大肠腺癌 Dukes ’ 分期(50.0 %，65.6 %，55.6 %)和淋巴结转移(64.0 % vs 57.1 %)无明显相关(P >0.05).

2.3 Survivin 表达与凋亡指数，Ki-67 标记指数的相关性比较大肠腺癌、腺瘤 Survivin 阳性表达组与阴性组中凋亡指数、Ki-67 标记指数，发现凋亡指数阳性组低于阴性组(P <0.01)，KI-67 标记指数阳性组高于阴性组(P <0.01，见表 1).

表 1 大肠腺瘤和腺癌中 Survivin 蛋白表达与凋亡指数、Ki-67 标记指数关系($\bar{x} \pm s$)

诊断	*n*	凋亡指数				Ki-67 标记指数			
		阳性(%)	*n*	阴性(%)	*n*	阳性(%)	*n*	阴性(%)	
大肠腺癌	6	1.15 ± 0.47	29	1.78 ± 1.12[a]	6	25.1 ± 7.6	29	19.7 ± 5.8[a]	
大肠腺瘤	36	0.85 ± 0.52	24	1.25 ± 0.58[a]	36	47.5 ± 11.3	24	34.4 ± 8.5[a]	

[a]P <0.01, vs 阳性.

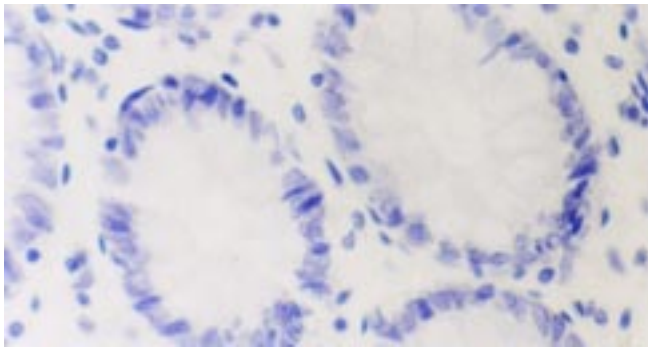

图1 Survivin在正常大肠黏膜组织表达S-P法× 400.

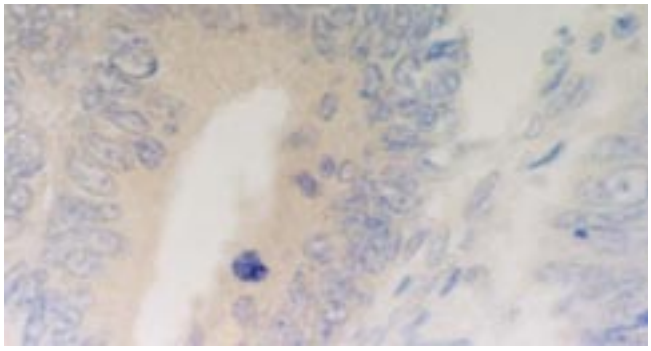

图2 Survivin在腺癌组织表达S-P法× 400

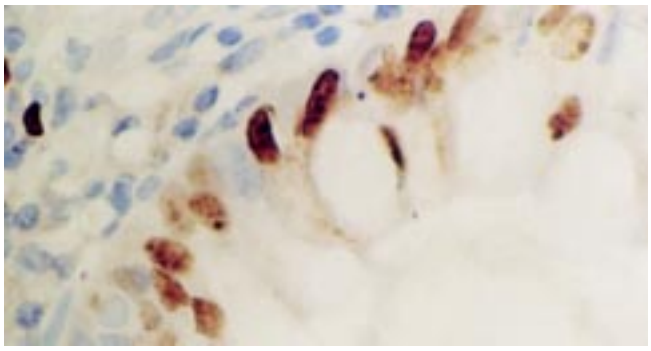

图3 Ki-67在腺癌组织表达S-P法× 400

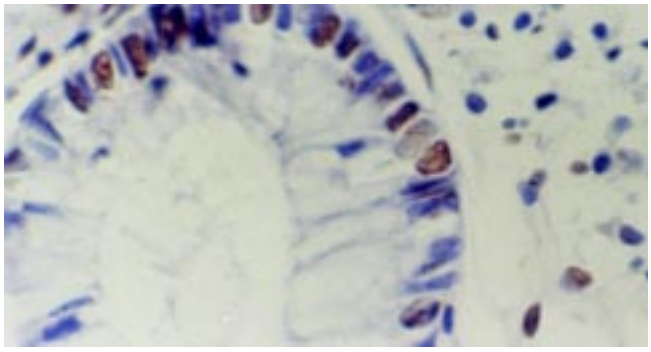

图4 高分化腺癌组织中凋亡细胞TUNEL法× 400

3 讨论

调节细胞凋亡的基因参与了肿瘤的发生发展过程，主要与三个基因家族即bcl-2家族、Caspase家族和IAP家族关系密切[22,23]. Survivin是IAP家族的新成员，结构独特，仅含有一个杆状病毒凋亡抑制蛋白重复序列(BIR)分子，作用于各种凋亡通路末端效应分子，是迄今发现的最强的凋亡抑制因子之一. 实验研究证实，Survivin能抑制Fas，Caspase，Bax及某些化疗药物诱导的凋亡[24-26]. 我们运用免疫组化方法显示Survivin蛋白在正常大肠黏膜中不表达，在腺瘤、腺癌组织中总表达率分别为17.1 %和60.0 %，提示Survivin蛋白过度表达是大肠肿瘤发生的早期事件，且具有促进腺瘤向腺癌转化作用，与Kawasaki et al [27]报道结果一致. Survivin作为广泛的肿瘤抗原，在抗肿瘤免疫治疗中有广阔前景[28,29]. Ki-67是非组蛋白成分的DNA结合蛋白，其表达程度可以反映细胞的增生活性. 我们发现在大肠腺癌中Ki-67标记指数高于腺瘤，Survivin阳性组高于阴性组，而凋亡指数阴性组高于阳性组($P < 0.01$)，进一步表明Survivin基因主要经过调控细胞凋亡和增生，参与大肠癌的发生和发展. 基础实验表明凋亡调控多数发生在G2/M期，Survivin-RNA在G2/M细胞表达上调40倍[30]. Survivin在细胞分裂过程中与细胞分裂微管结合控制其稳定性和有丝分裂纺锤体的聚集[31].因此，肿瘤组织可能通过对Survivin过量表达上调他们在有丝分裂中抗凋亡作用，保持肿瘤的增生和生长.Survivin基因有望成为治疗大肠癌一个较为理想的潜在靶点.

本研究显示Survivin蛋白在大肠腺癌高、中、低分化组中的表达率分别为45.5 %，52.2 %和73.1 %，3组间差异有显著性($P < 0.05$)，提示Survivin蛋白的表达率在一定程度上反映了腺癌的恶性程度，可以作为预后不良参数指标. Rodel et al [32]报道直肠癌高Survivin表达的患者5 a生存率明显低于低Survivin表达者(18 % vs 77 %)，肿瘤转移前者是后者的4倍(78 % vs 18 %)，认为Survivin阳性表达是直肠癌转移的高危因素. 亦有文献报道Survivin检测能预测II期结肠癌的发生，因而我们认为检测Survivin对预测肿瘤的发生，发展和预后提供了一新的指标. 我们还发现尽管Survivin在大肠腺癌中有过表达，但与Dukes'分期和淋巴结转移无明显关系，与国内学者在肺癌、子宫颈癌等研究结果一致[33,34]. 这提示在大肠癌进展过程中还受其他癌基因的调控，是多基因参与，多因素共同作用的结果[35,36]，Survivin基因与其他癌基因关系有待进一步研究.

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