G3BP 在结肠癌中表达的临床病理学研究

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摘要 目的：探讨 G3BP (Ras-GTPase-activiting protein SH3 domain binding protein) **在结肠癌组织中的表达及其临床病理意义。方法：用免疫组织化学技术检测** 50 **例结肠癌组织中** G3BP **的表达，并分析其与患者临床病理指标的关系及其对预后的影响。结果：** G3BP **在结肠癌中阳性表达率为** 72% (36/50)，G3BP **的阳性表达率与肿瘤分化程度、淋巴结转移、病变浸润层次及** TNM **分期有关。而与年龄、性别、肿瘤大小无关** (P＞0.05)。G3BP **高表达组患者的生存时间明显短于低表达组。结论：** G3BP **表达与结肠癌的侵袭和转移相关，可能是结肠癌的预后差的指标。**

关键词：结肠癌；G3BP**；免疫组织化学；预后；转移**

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A Clinicopathological Study on G3BP Expression in Colon Carcinoma

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ABSTRACT Objective: To investigate the expression of G3BP (Ras-GTPase-activiting protein SH3 domain binding protein) in colon cancer (CC) and its clinical pathological significance. Methods: The expression of G3BP was investigated by immunohistochemical staining in each 50 pathologically verified CC, and the relation to clinical pathological index was involved. Results: The G3BP positive expression rate in CC was 72% (36/50). The expression of G3BP was associated with tumor differentiation, lymph node metastasis, invasion depth and TNM stage, but not with age, gender, tumor diameter (P>0.05). The G3BP high expression group had a significantly shorter median survival time than the low expression group patients. Conclusion: G3BP is closely related to invasion and metastasis of CC, and may be an index for poor prognosis.

Key words: Colon cancer; G3BP; Immunohistochemistry; Prognosis; Metastasis

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前言

新近研究发现 G3BP 在多种肿瘤组织中呈高表达，具有抑制凋亡、促进肿瘤发生和发展作用。本研究应用免疫组织化学方法检测了 G3BP 在结肠癌组织的表达及临床病理学意义进行探讨。

1 材料和方法

1.1 病例资料

收集 2005 年内湖南省肿瘤医院普外科接受结肠癌根治手术的结肠癌标本 50 例。患者年龄分布在 34~81 岁之间，中位年龄 63.8 岁；其中男 32 例，女 18 例；伴有淋巴结转移者 35 例，不伴淋巴结转移者 15 例。均为散发性结肠癌，术前未行放疗及化疗；术后经病理确诊为腺癌，其中高分化或中分化腺癌 39 例，低分化或未分化腺癌 11 例。

1.2 试剂

兔抗人 G3BP(H-94)多克隆抗体购自美国 Santa Cruz 公司(sc-98561)；即用型 SP 免疫组化染色试剂盒和 DAB 显色试剂盒均购自福州迈新生物技术公司。

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1.3 免疫组化操作步骤

石蜡切片常规脱蜡脱水，3% H_2O_2 孵育 20min，PBS 洗，0.01 mol/L 枸橼酸盐缓冲液中微波处理进行抗原修复，PBS 洗；滴加封闭血清孵育 20 min，滴加一抗 4℃孵育过夜，G3BP 的工作浓度是 1:200，PBS 洗，滴加辣根过氧化物酶标记二抗抗体孵育 15min，PBS 洗；DAB 溶液显色；自来水冲洗，苏木素复染脱水封片，光镜下观察。结果判定根据胞浆出现的棕黄色颗粒样染色的细胞数和染色强度，采用 Sinicrope 等改良计分法判定阳性表现。具体计分方式如下：染色细胞计分：按照至少 5 个 400 倍视野下染色的细胞所占百分比分为 5 级，＜5%、5%～＜25%、25%～＜50%、50%～＜75%、≥ 75%分别计为 0、1、2、3、4 分；染色强度计分：弱染色、中度染色、强染色分别计为 1、2、3 分。每张切片总计分 = 染色细胞计分× 染色强度计分，总计分＜2 分为阴性，≥ 2 分为阳性。PBS 液代替一抗作阴性对照，已知 G3BP 阳性的组织切片作阳性对照。

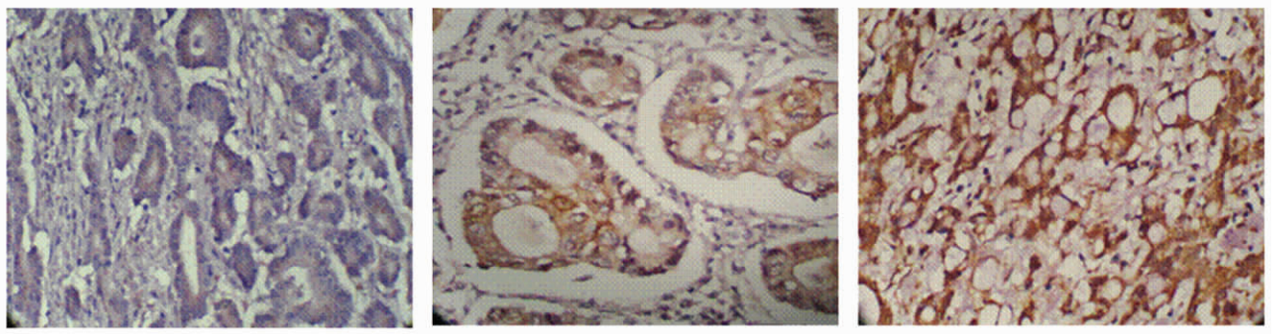
1.4 统计学方法

实验数据用 SPSS18.0 软件包进行统计学分析，通过 χ^2 检验比较阳性率间的差异，以 P＜0.05 作为有统计学意义。将有随访资料病例的随访时间按不同因素绘制生存曲线。检验水准为 $\alpha = 0.05$，P＜0.05 为差异有统计学意义。

2 结果

2.1 G3BP 在结肠癌中的表达

应用免疫组织化学方法检测结肠癌组织中 G3BP 蛋白表达情况，G3BP 阳性信号主要定位在癌细胞的胞浆，根据阳性信号的强弱在细胞浆内显示深浅不一的棕黄色到棕色颗粒状和片状信号(图 1)。G3BP 在 50 例结肠癌中的阳性表达率是 72%(36/50)。



高分化腺癌 High-differentiation adenocarcinoma　中分化腺癌 Mediate-differentiation adenocarcinoma　低分化腺癌 Low- differentiation adenocarcinoma

图 1 G3BP 在不同结肠癌中的表达情况

Fig.1 G3BP expression in colon carcinoma with different differentiation

2.2 G3BP 的表达与结肠癌临床病理特征的关系

50 例结肠癌中，G3BP 的阳性表达率与肿瘤分化程度、淋巴结转移、病变浸润层次及 UICC TNM 分期(均 P<0.05)有关，而与年龄、性别、肿瘤大小无关(P＞0.05)，侵犯肌层，浆膜层的病例组的阳性率显著高于侵犯粘膜层，粘膜下层病例组。高 - 中分化组的阳性率显著高于中 - 低分化组，III~ IV期组的阳性表达率显著高于 I ~ II 期组。有淋巴结转移组阳性表达率显著高于无淋巴结转移组。见表 2。

表 1 G3BP 在结肠癌的不同病理特征组中的表达

Table 1 G3BP expression in colon carcinoma with different pathological features

指标(Index)	G3BP 阳性 Positive	G3BP 阴性 Negative	阳性率 Positivity rate (%)	x^2	P
年龄 (岁) Age(years)				0.031	0.860
≥ 50	19	7	73.1		
＜50	17	7	70.8		
性别(Gender)				2.941	0.086
男(Male)	20	4	83.3		
女(Female)	16	10	61.5		
直径 (cm)				0.511	0.475
≥ 5	14	7	66.7		
＜5	22	7	75.8		
侵犯层次(Invasion depth)				5.821	0.016
粘膜层、粘膜下层 Mucosa, submucosa	6	7	46.2		
肌层、浆膜层 Muscularis, placenta percreta	30	7	81.0		
分化程度				2.131	0.044
高 - 中分化 High- mediate	30	9	76.9		
中 - 低分化	6	5	54.5		
UICC TNM 分期(Stage)				9.432	0.002
I ~ II	11	11	50.0		
III~ IV	25	3	89.3		
淋巴结转移 (Lymph node metastasis)				15.892	0.000
无 (No)	5	10	33.3		
有 (Yes)	31	4	88.6		

2.3 生存分析

全部结肠癌患者中位随访时间 53.5 个月 (20~81 个月)。G3BP 高表达组患者的生存时间 46.2 个月,明显短于低表达组的 64.5 个月(P=0.005)。

3 讨论

G3BP (Ras-GTPase-activiting protein SH3 domain binding protein) 是第一个被发现能够与 Ras-GAP SH3 结构域特异性结合的蛋白。在 Ras 信号转导途径中,G3BP 是一种 RasGAP SH3 结构域特异性结合蛋白,参与 Ras 下游信号通路,属于 RNA 结合蛋白家族。G3BP 具有核酸内切酶、DNA 解旋酶活性,能够诱导应急颗粒的形成,参与多种细胞生长、分化、凋亡和 RNA 代谢的信号通路[1-3]。

已经发现 G3BP 在多种肿瘤组织和细胞中高表达[4],并且与恶性肿瘤侵袭和转移相关。本研究发现 G3BP 阳性表达率与肿瘤侵犯肠壁深度,淋巴结转移以及肿瘤分化程度有关。侵犯肠壁越深,肿瘤分化越差以及有淋巴结转移的病例 G3BP 阳性表达率越高。有一些研究报道也支持这一结果,如在食道癌的研究中发现,G3BP 在淋巴结转移病例中的表达率显著高于无淋巴结转移组[5,6]。也有报道在胰腺癌细胞中 CD24 与 G3BP 有密切关系,二者形成复合物,CD24 可抑制 G3BP 的内切酶活性进一步调控 BART 表达,从而抑制癌细胞的侵袭和转移 ;而阻断 CD24 则增加胰腺 3BP 在肿瘤侵袭中发挥作用。G3BP 与临床分期和生存期长癌动物模型中腹膜后侵袭和肝转移。G3BP 氨基端区通过对 BART 的翻译后调节增加细胞运动和侵袭[7,8]。在乳腺癌 Her2 过表达病例中 G3BP 也呈雌激素非依赖性过表达,说明乳腺癌进展有关 [9]。在肺大细胞癌转移研究中发现,G3BP 在转移能力不同的细胞中表达强度有显著差别,在非转移细胞中呈强表达而在高转移性细胞株中呈弱表达,这样的现象也出现在前列腺癌细胞中。研究发现茶多酚 EGCG 通过与 G3BP1 结合来抑制肺癌发生[10,11]。

我们的临床病理研究也显示临床分期较晚病例组,G3BP 阳性表达率越高。而 G3BP 阳性病例的生存期明显短于阴性病例。在食道癌研究中也有与本研究一致的结论,即 G3BP 阳性病例的生存期比 G3BP 阴性病例更短,并且 G3BP 表达与 RhoC 表达呈正相关[4]。

关于 G3BP 影响恶性肿瘤进展的机制目前有一些报道,如 G3BP 可影响细胞周期和细胞增殖,其在肿瘤中表达增加提示其能促进瘤细胞增殖和去分化,并且促进纤维母细胞进入 S 期[12-14]。又如在肿瘤细胞中抑制 G3BP1 或 G3BP2 可引起 P53 显著上调[15]。正是由于 G3BP 在细胞增殖,分化,凋亡和 RNA 代谢相关信号通路中发挥作用,并且在许多恶性肿瘤中呈过表达且与肿瘤侵袭和转移有关,所以该因子可能成为肿瘤治疗的一种新靶分子[16-20]。对于 G3BP 在结肠癌中侵袭,转移和预后的相关机制有待细胞和分子生物学的进一步研究。

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