

Nucleophosmin/B23对结肠癌侵袭能力的影响*

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摘要 目的:探讨核仁磷酸蛋白(Nucleophosmin/B23)在人结肠癌组织中的表达及对人结肠癌细胞侵袭能力的影响。**方法:**选取2000年6月至2005年10月就诊于天津医科大学附属肿瘤医院结肠癌患者31例,并收集其肿瘤组织、对应的癌旁组织和转移淋巴结组织,采用免疫组织化学染色方法检测B23的表达情况。采用Western blotting技术检测不同结肠癌细胞系中B23的表达情况,利用小干扰RNA技术下调B23在结肠癌细胞中的表达,利用Transwell侵袭实验观察B23表达下降对结肠癌细胞侵袭能力的影响。**结果:**免疫组织化学染色显示B23在结肠癌组织的表达高于结肠癌旁组织表达($P=0.0016$),在转移淋巴结中的表达高于结肠癌旁组织($P=0.0007$),差异有统计学意义。Western blotting证实转染B23特异性小干扰RNA的结肠癌细胞HCT116中B23的表达明显下降,同时明显抑制结肠癌细胞的侵袭能力。**结论:**B23在结肠癌和转移淋巴结中高表达,且能影响结肠癌细胞的侵袭能力。提示B23可能在结肠癌的发生、进展和浸润转移中起调节作用。

关键词 Nucleophosmin/B23 结肠癌 HCT116 转移

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Effects of Nucleophosmin/B23 on Invasion of Human Colon Cancer

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Abstract Objective: To study the expression of B23 in human colon cancer tissues and its role on invasion in colon cancer cells. **Methods:** A total of 31 patients with colon cancer, who were admitted to the Tianjin Medical University Cancer Institute and Hospital from 2000 to 2005 were enrolled in the current study. Immunohistochemical assay was used to analyze the expression of B23 in colon cancer, para-neoplastic tissues of the cancer, and lymph nodes tissues. Statistical analysis of the immunohistochemical results was conducted using the SPSS 17.0 software. The expression of B23 in different colon cancer cell lines was determined by Western blot. In addition, the small interfering RNA (siRNA) technology was utilized to down-regulate the expression of B23 in the HCT116 cells. The invasive ability of the colon cancer cells was observed after the knock down of B23 via the Transwell assay. **Results:** Immunohistostaining results revealed that the expression of B23 was higher in colon cancer than in the paraneoplastic tissues. Statistically significant differences between the expression of B23 in the colon cancer and lymph nodes were noted and compared with the paraneoplastic tissues adjacent to the colon cancer. The P values were 0.0016 and 0.0007, respectively. Western blot assay confirmed that the B23-specific siRNA could effectively knock down the expression of B23, and the decrease of the B23 expression notably inhibited the invasive ability of the colon cancer cells. **Conclusion:** B23 showed high expression in colon cancer and lymph node metastasis. The knockdown of B23 can inhibit the migration capability of the colon cancer cells. B23 may play an important role in the progression and metastasis of colon cancer.

Keywords Nucleophosmin/B23; Colon cancer; HCT116; Metastasis

结肠癌是危害人类健康的常见消化道恶性肿瘤之一,占胃肠道肿瘤发病率的第3位。近年来,随着人们生活水平的提高,饮食结构的改变,其发病率呈逐年上升趋势。结肠癌的手术治愈率、5年生存率始终徘徊在50%左右,治疗失败的主要原因为局部复发率较高。国内目前的结肠癌靶向治疗药物比较单

一,单抗西妥昔(爱必妥)主要是针对EGFR的单克隆抗体。核仁磷酸蛋白(nucleophosmin/B23)可穿梭于核仁、核质和胞质之间,是一种多功能的细胞磷酸化蛋白,其参与核糖体的装配与合成、染色体和中心体的复制、维持细胞核和核仁的结构以及参与细胞内信号传导等,在肿瘤细胞和增生细胞中的含量明显

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高于正常静止期细胞,具有促进细胞增生和致癌基因的特性。B23的突变可以导致p53基因失活,c-myc稳定性增强,从而促进细胞发生恶性转化,甚至B23的突变可能是导致急性髓系白血病发生的起始事件^[1]。B23在多种实体肿瘤中过表达,甚至可作为胃、结肠、卵巢和前列腺肿瘤的标志物^[2-5]。但尚未见文献报道B23与结肠癌转移的关系。为探讨B23在结肠癌浸润转移中的作用,本研究观察了B23在结肠癌组织中的表达情况以及其表达下调对结肠癌细胞侵袭能力的影响,进一步探讨其相关机制。

1 材料与方法

1.1 细胞系和相关试剂

人结肠癌细胞系HCT116由本实验室保存。DMEM/F12、IMDM、胎牛血清(FBS)、胰酶均购自HyClone公司,小鼠抗人B23单克隆抗体和HRP标记山羊抗小鼠抗体均购自Santa Cruze公司,ECL化学发光底物购自Millipore公司。Matrigel胶购自美国BD公司,Transwell Cell Cultrue Insert购自Millipore公司,DAB显色液及一抗稀释液均购自中杉公司。

组织标本及病例:收集天津医科大学附属肿瘤医院病理档案资料中结肠手术病例31例,包括结肠癌组织、配对的癌旁组织及转移淋巴结组织。

1.2 免疫组织化学

将石蜡切片置于65℃烤箱12h,脱蜡返水后,采用枸橼酸盐缓冲液(pH=7.4)高压抗原修复2min,待温度降至室温后,采用3% H_2O_2 常温避光孵育30min以去除内源性过氧化物酶的影响,然后加入一抗(B23,1:50稀释),4℃孵育过夜,PBS洗3遍后加入通用二抗,37℃孵育30min,最后采用二氨基联苯胺(DAB)显色,苏木素轻度复染细胞核,脱水、透明、封片后在显微镜下观察。用已知阳性切片(乳腺癌)做阳性对照,阴性对照切片用PBS代替一抗。

1.3 细胞培养

人结肠癌细胞系HCT116细胞采用含20%胎牛血清、100 U/mL青霉素和100 U/mL链霉素的IMDM培养液,置于37℃,5% CO_2 的培养箱中培养,每2~3d传代1次。

1.4 细胞转染

将处于对数生长期的HCT116细胞按每孔 5×10^5 个细胞种于6孔板,待细胞生长至60%左右时,采用Lipofectamine™2000将化学合成的B23特异性小干扰siRNA转染入HCT116细胞中,序列为5'-CCU-AGUUCUGUAGAAGACATT-3'。转染48h后收获细胞,进行细胞学实验。

1.5 Western blot检测B23在结肠癌细胞中的表达水平
采用RIPA法提取细胞总蛋白,蛋白定量后进行

SDS-PAGE电泳并将蛋白转移至PVDF膜上;然后将膜在5%脱脂奶(用含0.05%吐温的TBS缓冲液即TBST配置)中封闭1h;加入鼠抗人B23单克隆抗体(1:2000)于4℃孵育过夜,采用TBST洗3次后加入HRP标记的羊抗鼠二抗(1:5000),并在水平摇床室温振摇1h,然后加入ECL化学发光剂(1:1)显色,胶片曝光检测蛋白表达水平。

1.6 细胞侵袭实验(invasion assay)

在冰上将Matrigel胶与无血清DMEM培养基以1:5比例稀释,然后在12孔transwell小室中每孔加入120 μ L,然后置于37℃孵育1h使基质胶凝固。消化重悬细胞并调整细胞浓度为 5×10^5 个/mL,将400 μ L细胞悬液加入Transwell上室,下室中加入800 μ L含10%血清的培养基,37℃孵育48h后,Matrigel胶,擦去未穿过膜的细胞,4%PFA(多聚甲醛)固定10min,然后采用0.005%结晶紫染色40min,200倍显微镜下观察透过膜的细胞数,随机选5个视野计数取平均值,并使用软件GraphPadPrim5作图。

1.7 统计学方法

所有统计分析采用SPSS 17.0统计软件进行,统计方法包括配对 χ^2 检验,t检验。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 B23在结肠癌组织,转移淋巴结组织和癌旁组织中的表达

200倍镜下观察,胞核中出现棕黄色颗粒为阳性。根据着色深浅分为:深棕色为强阳性(+++),棕黄色阳性(++),浅黄色为弱阳性(+),不着色为阴性(-)。结肠癌旁组织中B23阳性6例,结肠癌组织中阳性19例,转移淋巴结组织中B23表达阳性20例。在结肠癌组织和转移淋巴结中B23的表达高于对应的癌旁组织($P=0.0016$, $P=0.0007$)。见图1,表1。

2.2 不同结肠癌细胞系中B23的表达

Western blot结果表明B23在5种不同结肠癌细胞系中均表达,其中在低分化高转移细胞系HCT116中B23表达水平最高。见图2。

2.3 siRNA干扰后B23在HCT116细胞系中的表达水平

Western blot实验结果表明,与正常HCT116细胞和对照组Scr对比,siRNA干扰后的HCT116细胞B23表达水平明显降低。见图3。

2.4 B23的表达对HCT116细胞侵袭能力的影响

细胞侵袭实验表明HCT116细胞穿透Matrigel胶的能力明显高于小RNA干扰B23表达后的HCT116细胞,且差异有统计学意义($P < 0.001$)。见图4。

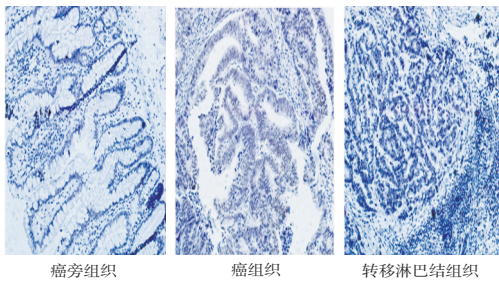


图1 B23在结肠癌组织、癌旁组织和转移淋巴结组织中的表达 (HE × 200)

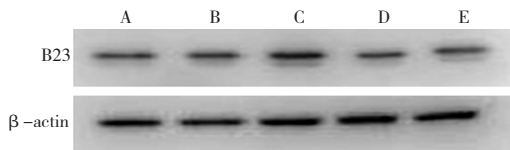
Figure 1 Expression of B23 in colon cancer, paraneoplastic tissues, and lymph node metastasis (H&E × 200)

表1 B23在不同组织中表达的比较 例(%)

Table 1 Statistical analysis of B23 expression in colon cancer, paraneoplastic tissues, and lymph nodes

项目	例数	B23表达	
		阴性	阳性
结肠癌组织	31	12(38.7)*	19(61.3)
转移淋巴结组织	31	11(35.5) [#]	20(64.5)
结肠癌旁组织	31	25(91.9)	6(8.1)

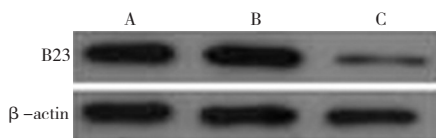
*与癌旁组织对比, $P=0.0016$, [#]与癌旁组织对比, $P=0.0007$



A: SW480; B: Colo205; C: HCT116; D: SW620; E: HT29

图2 B23在不同结肠癌细胞系中的表达

Figure 2 Expression of B23 in different colon cancer cell lines



A: HCT116; B: Scr/HCT116; C: SiB23/HCT116

图3 siRNA干扰后B23在HCT116细胞系中的表达

Figure 3 Detection of B23 expression using the Western blot method

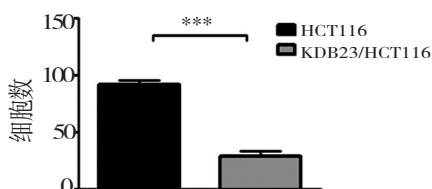
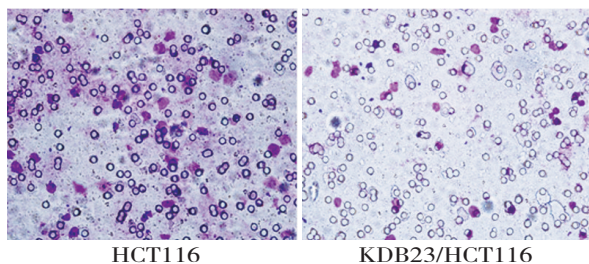


图4 抑制B23后对HCT116细胞侵袭能力的影响(×200)

Figure 4 Knockdown of B23 decreases the invasion ability of HCT116 cells

3 讨论

结肠癌是消化道最常见的恶性肿瘤之一,浸润和转移是结肠癌等恶性肿瘤生物学行为的具体反映。其有效的治疗方法仍然是手术治疗和化学治疗,但由于较高的复发率,结肠癌患者5年生存率仍不乐观。近年来国外研究表明B23在多种肿瘤组织呈高表达,如胃癌^[6]、结直肠腺瘤^[7]、前列腺癌^[8]和膀胱癌^[9]等肿瘤组织中表达均增高。在多种肿瘤中,B23的表达水平与肿瘤发展阶段呈正相关^[10],如B23 mRNA的过表达与膀胱癌的复发及向更晚期发展相关^[11]。此外,B23作为雌二醇调控的蛋白质与人乳腺癌细胞获得雌二醇非依赖性有关。这些结果表明B23具有癌基因的功能。研究发现B23的缺失、突变甚至重排与多种肿瘤的发生密切相关^[12-13]。这表明B23的作用及其机制非常复杂,可能通过多种机制调控肿瘤的发生。所以本课题主要研究B23与肿瘤转移的相关性,为临床靶向治疗提供理论依据。

本研究通过免疫组织化学实验结果显示在31例结肠癌组织、配对的淋巴结组织和结肠癌旁组织中,结肠癌组织中B23阳性19例,转移淋巴结组织中B23阳性20例,结肠癌旁组织中B23阳性6例,提示B23在结肠癌组织中表达明显高于癌旁组织,差异统计有意义($P=0.0016$),且在转移淋巴结组织中表达明显高于癌旁组织,差异有统计学意义($P=0.0007$)。

同时发现B23在结肠癌不同细胞系中的表达水平不同,在高转移性低分化细胞系HCT116中表达最高。肿瘤细胞在原发部位突破基底膜,进而侵袭周围组织甚至引起远处转移^[14],众所周知,肿瘤细胞运动能力越强,转移发生越早,预后越差。我们选择在结肠癌细胞系HCT116中应用小RNA干扰技术降低B23的表达,通过细胞侵袭实验证明了降低B23的表达明显抑制了HCT116细胞的侵袭能力。提示B23与结肠癌的运动和转移密切相关,Bar图显示差异有统计学意义($P<0.001$)。

综上所述,B23是一个具有复杂功能的蛋白,通过B23在结肠癌中的研究,推测B23参与调节结肠癌转移的机制,B23的深入研究将为相关肿瘤的治疗提供新的思路。

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