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醛酮还原酶家族 1 的 C1 亚型在胃癌的表达及其作用探讨 *

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摘要 目的:研究 AKR1C1(Aldo-keto reductase family 1 member C1)在胃癌中的表达,并初步探讨其在胃癌发生和发展中的作用。**方法:**采用胃癌组织芯片和免疫组化分析方法,检测 60 例胃癌患者癌组织及癌旁正常组织 AKR1C1 的表达;实时荧光定量 PCR 和蛋白免疫印迹 western blot 检测胃癌细胞株 SGC-7901 AKR1C1 的表达;在胃癌细胞株 SGC-7901 中转染沉默 AKR1C1 的 shRNA 质粒及空质粒,MTT 比色法检测各实验组细胞增殖。**结果:**组织芯片和免疫组化结果显示,与正常组织相比,胃癌组织中 AKR1C1 呈高表达;实时荧光定量 PCR 和 western blot 观察可以发现胃癌细胞株 SGC-7901 高表达 AKR1C1;MTT 比色法检测发现,转染沉默 AKR1C1 的 shRNA 质粒组与空质粒对照组相比,SGC-7901 细胞的增殖受到明显抑制,差异有统计学意义($P < 0.05$)。**结论:**AKR1C1 与癌细胞的增殖有关,可能是其参与或间接参与了癌细胞的生长周期,为胃癌的发生及细胞增殖提供了新的研究思路 and 方向。

关键词:AKR1C1;SGC-7901 细胞;增殖

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Preliminary Discussion on the Expression and the Effect of Aldo-keto Reductase Family 1 Member C1 in Gastric Carcinoma*

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ABSTRACT Objective: To investigate the expression of AKR1C1 (aldo-keto reductase family 1 member C1) in gastric carcinoma and discuss its role in gastric carcinogenesis and development. **Methods:** Gastric cancer tissue microarray and immunohistochemical method were used to detect the expression of AKR1C1 in gastric carcinoma tissue microarray of 60 patients. Use the method of real-time fluorescence quantitative PCR and protein western blot to detect the expression of AKR1C1 in gastric cancer cell lines SGC-7901. Transfect gene silencing AKR1C1 short hairpin RNA plasmid and empty plasmid in gastric cancer cell lines SGC-7901 and use the method of MTT colorimetric assay to detect cell proliferation. **Results:** Tissue microarray and immunohistochemical method showed that the expression of AKR1C1 in the cancer tissue was higher than that in normal tissue. High expression of AKR1C1 in gastric cancer cell lines SGC-7901 was detected by the method of real-time fluorescence quantitative PCR and protein western blot. The method of MTT colorimetric assay found that cell proliferation was significantly inhibited in gene silencing AKR1C1 short hairpin RNA plasmid transfection group, compared with empty plasmid and blank group. And the difference has statistical significance($P < 0.05$). **Conclusions:** AKR1C1 has relationship with proliferation of cancer cell. The reason may be that AKR1C1 is directly or indirectly involved in the growth cycle of cancer cell. Provide new research ideas and directions for gastric cancer proliferation occurs.

Key words: AKR1C1; SGC-7901 cell; Proliferation

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前言

癌症是发达国家的首要死亡原因,在发展中国家是死亡的第二大原因,其中胃癌的发病率极高,在 2012 年有近 100 万个

胃癌新发病例,占有癌症病例的 6.8%^[1],中国的胃癌发病率位居世界前列,在消化道肿瘤中排第一位^[2]。醛酮还原酶家族(Aldo-keto reductases AKRs)是一个含有 16 个家族的超家族,目前发现其家族成员超过 190 个,它们以烟酰胺腺嘌呤二核苷

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磷酸(triphosphopyridine nucleotide NADPH) 为辅酶,参与氧化还原反应^[3,4],其中,家族1的 AKR1C1-AKR1C4 亚型主要参与类固醇激素的代谢^[5],类固醇激素代谢失衡则会引起依赖此类激素的器官产生病变,如卵巢,乳腺,子宫,前列腺等。近年研究表明,AKR1 与肿瘤的关系成为研究的热点^[6-10]。本文主要观察醛酮还原酶家族1的 C1 亚型(AKR1C1)在胃癌组织中的表达情况,研究其在胃癌细胞中的作用,并对其机制进行初步探讨。

1 材料与方法

1.1 标本及试剂

胃癌组织芯片2张共60例,胃癌组织芯片上各标本均为病理医师确诊为胃癌,按照WHO(2010)对消化胃部肿瘤的分级标准进行分级,其中高分化21例,中分化19例,低分化20例,各病例癌旁正常胃组织60例。上海市长征医院肿瘤科惠赠,免疫组化试剂盒(福州迈新公司),人胃癌细胞株SGC-7901上海市长征医院肿瘤科惠赠,1640培养基(吉诺生物技术有限公司),胎牛血清(Gibco公司),RNAiso Plus(TAKARA公司),逆转录试剂盒(TAKARA公司),Lipofectamine 2000(invitrogen公司),沉默AKR1C1的shRNA质粒及空载质粒(上海吉凯生物有限公司),AKR1C1和actin引物(上海生工生物工程有限公司合成),BCA蛋白试剂盒(Thermo公司),化学发光显色液(Thermo公司),人AKR1C1单克隆抗体和人 β -actin单克隆抗体(abcam公司)。

1.2 实验方法

1.2.1 免疫组化 组织芯片免疫组化染色采用SP法,一抗为鼠抗人AKR1C1蛋白单克隆抗体,工作浓度为1:50。用磷酸盐缓冲液(PBS)替代一抗做阴性对照。阳性结果判断标准:AKR1C1在组织的细胞浆出现棕黄色颗粒为阳性表达,分别于低中高倍镜下观察。

1.2.2 细胞培养 人胃癌细胞株SGC-7901常规复苏,用含10%胎牛血清和1%双抗的1640培养液常规培养于37℃、5%CO₂的细胞培养箱内,待细胞单层贴壁生长至80%~90%融合度时,0.25%胰蛋白酶消化传代,取对数生长期细胞用于实验。

1.2.3 细胞瞬时转染 转染前一天,胰酶消化细胞并计数,将细胞铺板在1 mLmL含血清不含抗生素培养基的六孔板中培养;第二天,用无血清培养基分别稀释对照组、shRNA质粒组和Lipofectamine 2000转染试剂,质粒:Lipofectamine 2000为1:4,将实验分为空白组、空质粒转染组和shRNA质粒组,分别将各实验组质粒和转染试剂两两轻轻混匀,于室温放置20 min;6孔板各空中加入1 mLmL无血清培养基,将复合物加入到每孔中,摇动培养板,轻轻混匀,置于37℃、5%CO₂培养箱中培养6 h后,将无血清培养基更换为含有血清无双抗的培养

基,继续孵育96 h,于荧光显微镜下观察结果。

1.2.4 RT-PCR 检测 使用RNAiso Plus试剂提取各实验组细胞的总RNA,测定RNA含量,1%琼脂糖凝胶电泳观察所提取的总RNA纯度,再用逆转录试剂盒将总RNA逆转录合成cDNA;PCR引物序列如下: β -actin:上游引物5'-TGACGTGGACATCCGCAAAG-3',下游引物5'-CTGGAAGGTGGACAGC-GAGG-3'(205bp);AKR1C1:上游引物5'-GTAAAGCTTTA-GAGGCCAC-3',下游引物5'-ATAAGGTAGAGGTCAA-CATAA-3'(253bp)。操作步骤和加样体系按实时荧光定量试剂盒说明进行,荧光定量PCR仪进行聚合酶链式反应,计算各实验组AKR1C1相对表达量。

1.2.5 Western blot 检测蛋白表达 用RIPA蛋白裂解液提取各实验组细胞的总蛋白,用BCA法测定蛋白浓度,取20 μ g蛋白样品进行SDS-PAGE电泳,然后将蛋白转至硝酸纤维素膜(PVDF)上,再用5%脱脂牛奶室温封闭1 h,加入一抗工作液(β -actin 1:1 000,AKR1C1 1:1000),4℃摇床孵育过夜,第二天加入HRP标记的二抗(1:1 000),室温摇床孵育1 h,加化学发光显色液,凝胶成像分析系统显色观察。

1.2.6 MTT 比色法检测细胞增殖 将各实验组细胞分别消化铺在96孔板中,每孔细胞为 0.5×10^4 个,每组五个副孔,继续培养12 h,在每孔中加入20 μ L 5 $\times$ 的MTT溶液,在细胞培养箱中继续培养4 h,弃去培养基,加入150 μ L二甲亚砜(DMSO)溶液,平板振荡器震荡1 min,用酶标仪在570 nm处检测吸光度值;依照上诉方法分别检测12 h、24 h、48 h和96 h时各组的吸光度值;计算各实验组增殖率,细胞增值率=(实验组-对照组)/实验组,细胞增值抑制率=1-(实验组-对照组)/实验组。

1.3 统计学方法

应用统计学软件SPSS 21.0进行数据统计学分析,计量资料用mean $\pm$ SD表示,组间比较采用方差分析,P<0.05为差异有统计学意义。

2 结果

2.1 免疫组化

组织芯片免疫组化结果显示,癌旁正常组织AKR1C1表达量较低,高、中、低分化组表达AKR1C1的量与正常癌旁组织均有差异(P<0.05),高分化组与中分化组表达量差异无统计学意义(P=0.293),高分化组与低分化组表达量差异有统计学意义(P=0.002),中分化组与低分化组表达量差异有统计学意义(P=0.021),随着胃癌的分化程度越低,AKR1C1的表达量越高(P<0.05),见表1、图1。

表1 不同病理分级的胃癌组织中AKR1C1蛋白表达水平的比较

Table 1 Comparison of the expression of AKR1C1 in gastric carcinoma with different pathological grades

Group	Case	AKR1C1 expression level			
		-	+	++	+++
peri-tumorous normal tissues	60	5	51	4	0
Highly differentiated group	21	0	8	12	1
Moderately differentiated group	19	0	2	13	4
Poorly differentiated group	20	0	0	8	12

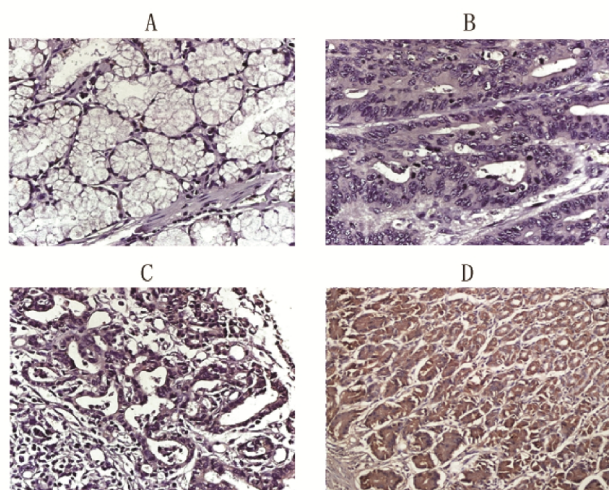


图1 AKR1C1在癌旁正常组织及胃癌组织中的表达(SP×400)

A.正常胃组织 B.高分化 C.中分化 D.低分化

Fig. 1 The expression of AKR1C1 in peri-tumorous normal tissues and gastric carcinoma (SP×400)

A. peri-tumorous normal tissues B. well differentiated C. moderately differentiated D. poorly differentiated

In peri-tumorous normal tissues, the amount of AKR1C1 is small, while it is larger in the cancer tissues. with the degree of differentiation reducing, the expression of AKR1C1 increases

2.2 细胞转染和检测

分别转染空的对照质粒和沉默 AKR1C1 的 shRNA 质粒, 提取各实验组的总 RNA 和总蛋白进行 RT-PCR 和 Western blot 验证见图 2, 空白组、空质粒转染组和 shRNA 质粒组

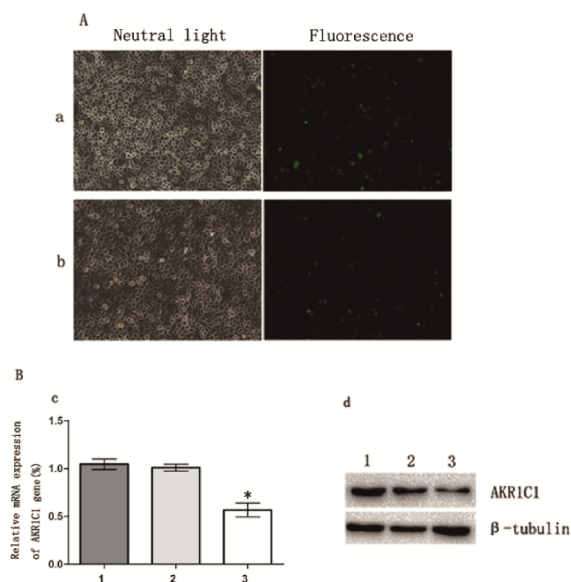


图2 转染效果及效率验证 A 质粒转染细胞效果图 a.转染空质粒细胞($\times 100$) b.转染 shRNA 质粒组细胞($\times 100$) B 质粒转染细胞效果验证 1.空白组细胞 2.空质粒组 3. shRNA 质粒组 c.AKR1C1 基因相对表达量 * $P<0.05$ d. AKR1C1 蛋白表达量,转染 shRNA 质粒组 AKR1C1 的蛋白表达量明显降低

Fig. 2 The transfection efficiency of verification A The effect of transfection empty a. transfection empty plasmid($\times 100$) b. transfection shRNA plasmid($\times 100$) B Transfection efficiency 1. Blank 2.empty plasmid 3. shRNA plasmid c. relative mRNA expression of AKR1C1 gene (%)* $P<0.05$ d. expression of AKR1C1 protein and β -tubulin detected by western blot. Transfection shRNA plasmid protein expression was significantly lower than other group

AKR1C1 的 mRNA 相对表达量分别为 (1.05 ± 0.07)、(1.00 ± 0.07)和(0.58 ± 0.01),沉默组表达量明显低于空白组和空质粒转染组, 两两组间比较, $P<0.05$ 差异有统计学意义。各组 AKR1C1 蛋白表达量分别为 (1.15 ± 0.02)、(1.00 ± 0.01)和(0.48 ± 0.01), 沉默组蛋白表达量明显低于空白组和空质粒转染组, 两两组间比较, 差异有统计学意义($P<0.05$)。

2.3 对 SGC-7901 细胞增殖的影响

在转染 shRNA 质粒后, SGC-7901 细胞的增殖受到明显抑制, 见图 3, 差异有统计学意义($P<0.05$), 提示 AKR1C1 与细胞的增殖有关。

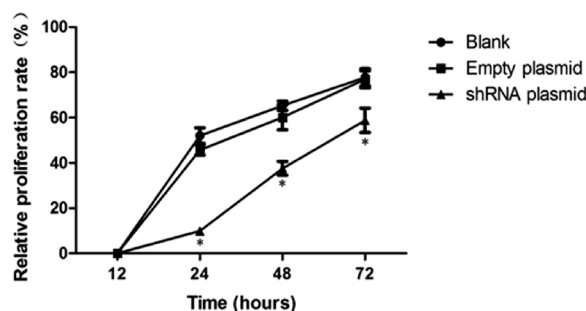


图3 空白组,空质粒组和 shRNA 质粒组细胞增殖情况

Fig. 3 Proliferation of blank, empty plasmid and shRNA plasmid

3 讨论

醛酮还原酶超家族(AKRs)是参与氧化还原反应的三大酶类超家族之一,近年来,关于醛酮还原酶家族1与肿瘤的发生、发展、复发及耐药成为研究热点;AKR1C1是醛酮还原酶家族1的C1亚型,存在于哺乳动物中,其分布和作用有组织特异性,在卵巢癌、乳腺癌中低表达^[11,12],而在非小细胞肺癌、肝癌中高表达^[13,14],相关报道显示,其表达的高低与疾病进程、复发、远处转移及预后有关^[15-17],也可以作为肺癌、前列腺癌、乳腺癌、膀胱癌等的新的治疗靶点^[18,19]。同时,AKR1C1也参与药物代谢,它的高表达与多种化疗药物耐受有关,包括柔红霉素、奥沙利铂、顺铂等^[20-22]。

本实验研究表明,AKR1C1在胃癌组织中的表达量比正常胃组织表达量高,且表达量与分化程度成反比,即 AKR1C1 的表达量越高,癌症的分化程度越低;通过 MTT 细胞增殖实验我们发现,转染了沉默 AKR1C1 的 shRNA 质粒组 SGC-7901 细胞生长速度显著受抑制,提示 AKR1C1 与细胞的增值有关。

真核生物细胞增殖主要方式是有丝分裂,这种方式主要包括两个阶段即分裂间期和分裂期,这两个阶段循环进行形成细胞周期,其中 DNA 的复制和蛋白合成都在分裂间期完成;当外来因素或物质影响任何一个阶段的任何一点都可能影响细胞的增殖。在本实验中,转染敲除 AKR1C1 的重组质粒胃癌细胞增殖率降低,这提示 AKR1C1 直接或间接参与细胞周期,但其具体机制和作用靶点仍不清楚,有待我们后续的实验进一步研究与证实。近年来,通过基因芯片技术及蛋白芯片技术我们确定了 AKRs 的不同亚型在不同肿瘤组织及细胞中的表达,同时通过基因缺失、异源表达以及表达模式研究等方法来推测这些酶在细胞代谢过程中的作用,但仍然不能窥其具体机制,有待深入研究。

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