

doi: 10.13241/j.cnki.pmb.2018.02.004

OIP5 对肝癌细胞 SMMC-7721 增殖和侵袭迁移能力的影响 *

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摘要 目的:探讨 OIP5 对肝癌细胞 SMMC-7721 增殖和侵袭迁移能力的影响。**方法:**采用 RNA 干扰技术沉默肝癌细胞中 OIP5 的表达后,通过 qRT-PCR 和 Western-blot 技术检测 OIP5 的下调效率,CCK-8 和平板克隆法检测肝癌细胞的增殖能力,Transwell 法检测肝癌细胞的侵袭和迁移能力。**结果:**转染 OIP5-siRNA 后,肝癌细胞 SMMC-7721 中 OIP5 mRNA 和蛋白的表达水平均明显降低($P<0.05$);同时,与对照组相比,OIP5-siRNA 组肝癌细胞 SMMC-7721 的 CCK-8 实验的 OD 值、平板克隆法测得的克隆球个数、Transwell 法测得的迁移细胞数与侵袭细胞数均明显低于对照组($P<0.05$)。**结论:**OIP5 能够促进肝癌细胞的增殖和侵袭迁移,可能作为肝癌治疗的潜在靶点。

关键词:OIP5;原发性肝癌;增殖;侵袭;迁移

中图分类号:R-33; R735.7 **文献标识码:**A **文章编号:**1673-6273(2018)02-215-04

Effects of OIP5 on the Proliferation, Migration and Invasion of Hepatocellular Carcinoma Cell line SMMC-7721 *

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ABSTRACT Objective: To investigate the protective effects of OIP5 on the proliferation, migration and invasion of hepatocellular carcinoma (HCC) cell line SMMC-7721. **Methods:** OIP5 was down-regulated by using small interfering RNA in HCC cell line SMMC-7721. The transfection efficiency of OIP5-siRNA was detected with qRT-PCR and Western blot. The proliferation of HCC cells was detected by CCK-8 and colony formation assay, and the migration and invasion of HCC cells were measured by Transwell assay. **Results:** The level of OIP5 mRNA and protein were significantly decreased after being transferred by OIP5-siRNA in SMMC-7721 cells. After knockdown of OIP5, the OD value measured by CCK-8, the number of colony, metastasis and invaded cell number were significantly decreased compared with the control group ($P<0.05$). **Conclusions:** OIP5 can promote the proliferation, invasion and metastasis of HCC SMMC-7721 cells, and targeting OIP5 is likely to be a promising strategy for the treatment of HCC.

Key words: OIP5; Hepatocellular carcinoma; Proliferation; Migration; Invasion

Chinese Library Classification(CLC): R-33; R735.7 **Document code:** A

Article ID: 1673-6273(2018)02-215-04

前言

原发性肝细胞癌(Hepatocellular carcinoma, HCC)是世界上第六大恶性肿瘤,中国每年有一半以上的新增或死亡病例与肝癌相关^[1]。最新数据显示中国肝癌患者的发病率和死亡率分别居中国恶性肿瘤的第四位和第三位^[2]。随着肝癌诊断和治疗手段的不断提高,肝癌患者的总体生存率仍然不佳^[3]。

OIP5 (Opa interacting protein 5) 是 CTA (cancer-testis antigen, 癌-睾丸抗原)家族成员之一,于 1988 年由 WILLIAMS 等^[4]运用酵母双杂交技术,以 OPa 蛋白为诱饵鉴定发现。研究显示 OIP5 在乳腺癌^[5,6]、肺癌^[7]、食管癌^[7]、胃癌^[8]、结肠癌^[9]和透明细

胞癌^[9]等多种肿瘤中表达上调,沉默 OIP5 基因后能够抑制多种肿瘤的增殖能力,提示 OIP5 可能是肿瘤治疗的一个潜在靶点,但目前 OIP5 在肝癌中的作用尚不完全明确。因此,本研究采用 siRNA 技术干扰 OIP5 肝癌细胞 SMMC-7721 中的表达,后通过 CCK-8、平板克隆和 Transwell 探讨了其对肝癌细胞的增殖和侵袭迁移能力的影响,以期对肝癌的治疗提供相关理论依据。

1 材料与方法

1.1 材料

人肝癌细胞系 SMMC-7721 (上海复旦大学中山医院肝癌

* 基金项目:国家自然科学基金项目(81672339)

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(收稿日期:2017-06-23 接受日期:2017-07-18)

研究所);OIP5-siRNA 和阴性对照(上海吉码公司);DMEM 培养基、胎牛血清(Gibco 公司);转染试剂 lipofectamine 2000(In-vitrogen 公司);Transwell 小室、Matrigel 胶(Corning 公司);OIP5 引物(上海生工生物公司);反转录试剂盒和定量试剂盒(Takara 公司);CCK-8 试剂盒(碧云天);OIP5 抗体(Proteintech 公司)。

1.2 方法

1.2.1 细胞培养与转染 SMMC-7721 细胞用完全培养基(含 10%胎牛血清的 DMEM 培养基)常规培养于 5%CO₂、37℃培养箱中。转染前将细胞接种到六孔板中,次日细胞密度达到 40%进行 siRNA 转染。每孔转染体系为 5 μL OIP5-siRNA+10 μL lipofectamine 2000。具体转染操作按 lipofectamine 2000 说明书进行。分别在细胞转染 24 h 和 48 h 后,收集细胞用于提取总 RNA 和蛋白。

1.2.2 Real-time PCR 方法检测 OIP5 mRNA 的表达 用 Trizol 常规提取各组细胞总 RNA,随后反转录成为 cDNA,以 β-actin 为内参,用荧光定量 PCR 仪进行扩增并采用 2^{-ΔΔCT} 法进行相对定量分析。实验步骤按照试剂盒说明书进行。OIP5 的引物序列,上游引物:CTTGTGGGATTCCCGTTGGTT;下游引物:TGTGCGTTAGCACTATCTTCTCT。

1.2.3 Western blot 方法检测 OIP5 蛋白的表达 利用 RIPA 蛋白裂解液冰上裂解各组细胞 15 min,振荡,离心后收集上清即为总蛋白。利用 BCA 蛋白定量试剂盒检测蛋白总浓度。将 30 μg 蛋白加入配制好的 SDS PAGE 凝胶上层胶加样孔中,常规电泳(上层胶分离条件为恒压 80 V 30 min,下层胶为恒压 120 V 60 min)分离后转至 PVDF 膜,随后利用 5%脱脂奶粉封闭 PVDF 膜 1 h,4℃冰箱孵育一抗(OIP5、β-actin)过夜。次日,回收一抗并用 TBST 洗 PVDF 膜 3 次后室温孵育二抗(HRP 标记)1 h。随后 TBST 洗 3 次,增强化学发光法显影。

1.2.4 CCK-8 细胞增殖实验 细胞转染 24 h 后,胰蛋白酶消化细胞,离心后重悬并调整细胞浓度为 2×10⁴/ml,以每孔 100 μl 接种于 96 孔板中,每组设置 5 个复孔。分别于 24 h、48 h、

72 h、96 h 和 120 h 进行 CCK-8 法检测。检测前按 1:10 的比例将 CCK-8 和完全培养基混合,每孔加入混合液 100 μl,继续培养 2 h 后,酶标仪 490 nm 波长检测各组吸光值(OD 值)。

1.2.5 平板克隆实验 细胞转染 24 h 后,胰蛋白酶消化细胞,离心重悬后计数。向 6 孔板每孔加入 300 个细胞,常规培养 14 天。PBS 洗一遍,4%多聚甲醛固定 15 min,0.4%结晶紫溶液染色 15 min,拍照并统计克隆球数量。

1.2.6 细胞侵袭迁移实验 细胞迁移实验:细胞转染 24 h 后,胰蛋白酶消化细胞,PBS 洗 1 遍,用无血清 DMEM 培养液重悬,调整细胞密度至 1.5×10⁵/mL,按 200 μL/孔加入到小室上层,小室下层加入 500 μL 含有 20%胎牛血清的 DMEM 培养基。培养 24 h 后,对小室下层的细胞用 4%多聚甲醛固定 15 min,0.4%结晶紫溶液染色 15 min 后,擦除小室膜上面的细胞,显微镜下每个小室随机取 5 个视野进行计数。细胞侵袭实验:实验前首先将把无血清 DMEM 按照 1:8 稀释的 100 μL Matrigel 胶加入到小室上层,其余步骤同前。

1.3 统计学分析

统计分析采用 SPSS 19.0 软件进行。数据以均数±标准差表示,两组间比较检验采用 t 检验,以 P<0.05 为差异具有统计学意义。

2 结果

2.1 在肝癌细胞 SMMC-7721 中下调 OIP5 的表达

采用 lipofectamine 2000 分别将 OIP5-siRNA 和阴性对照 OIP5-NC 转入到 SMMC-7721 细胞中,24 h 后提取 RNA,48 h 后收集蛋白。采用 Real-time PCR 和 Western blot 方法检测 OIP5 的沉默效率。结果显示:转染 OIP5-siRNA 的 SMMC-7721 细胞的 OIP5 mRNA 和蛋白表达水平明显低于阴性对照组(图 1A-B),提示转染 OIP5-siRNA 能够成功下调肝癌细胞中 OIP5 的表达。

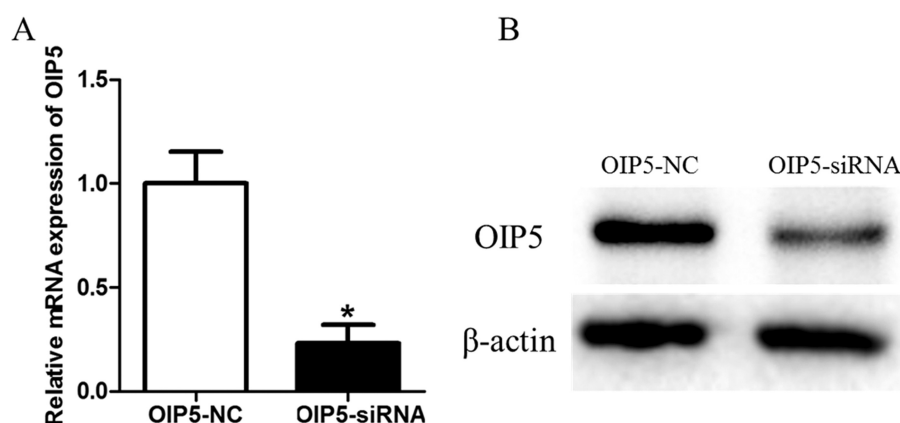


图 1 转染 OIP5-siRNA 后 SMMC-7721 细胞中 OIP5 mRNA 和蛋白的表达

Fig.1 OIP5 mRNA and protein expressions in the SMMC-7721 cells transfected with OIP5-siRNA

2.2 OIP5 表达下调对肝癌细胞 SMMC-7721 增殖能力的影响

CCK-8 法检测出的 OD 值能够反映各组细胞的数量和增殖能力的改变,并利用 OD 值绘制细胞生长曲线。结果显示,与对照组相比,OIP5-siRNA 组肝癌细胞 SMMC-7721 的 OD 值均

明显下降(P<0.05,图 2A)。采用平板克隆实验检测各组细胞克隆成球能力,结果显示:OIP5-siRNA 组肝癌细胞 SMMC-7721 的克隆球个数(48±5)明显低于对照组(85±8)(P<0.05,图 2B)。以上结果提示在肝癌细胞 SMMC-7721 中下调 OIP5 的表达能

够抑制其增殖能力。

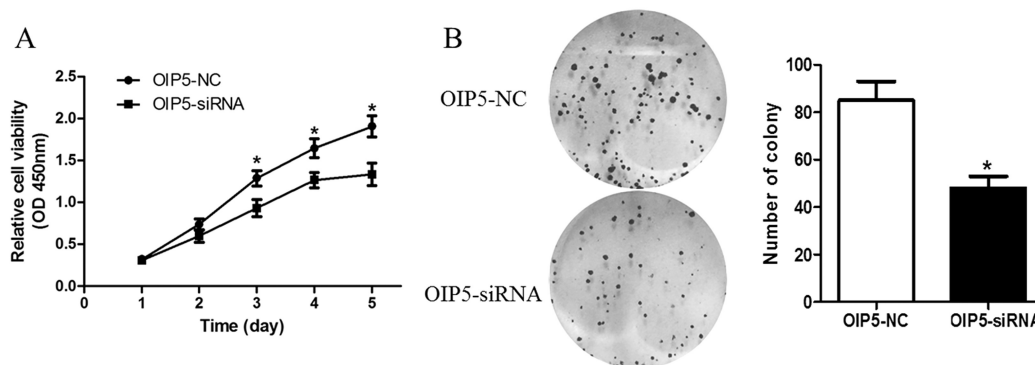


图2 转染 OIP5-siRNA 对 SMMC-7721 细胞增殖能力的影响

Fig.2 The effect of transfection with OIP5-siRNA on the cell proliferation ability of SMMC-7721 cells

2.3 OIP5 表达下调对肝癌细胞 SMMC-7721 侵袭迁移能力的影响

采用 Transwell 实验检测 OIP5 对肝癌细胞 SMMC-7721 侵袭迁移能力的影响,结果显示:高倍视野下对照组迁移的细胞数量为 (288±23) 个,侵袭的细胞数量为 (196±17),而

OIP5-siRNA 组迁移的细胞数量为(161±15)个,侵袭的细胞数量为(138±12)个(图 3A-B),均较对照组显著降低($P<0.05$)。以上结果提示在肝癌细胞 SMMC-7721 中下调 OIP5 的表达能够抑制肝癌的侵袭迁移能力。

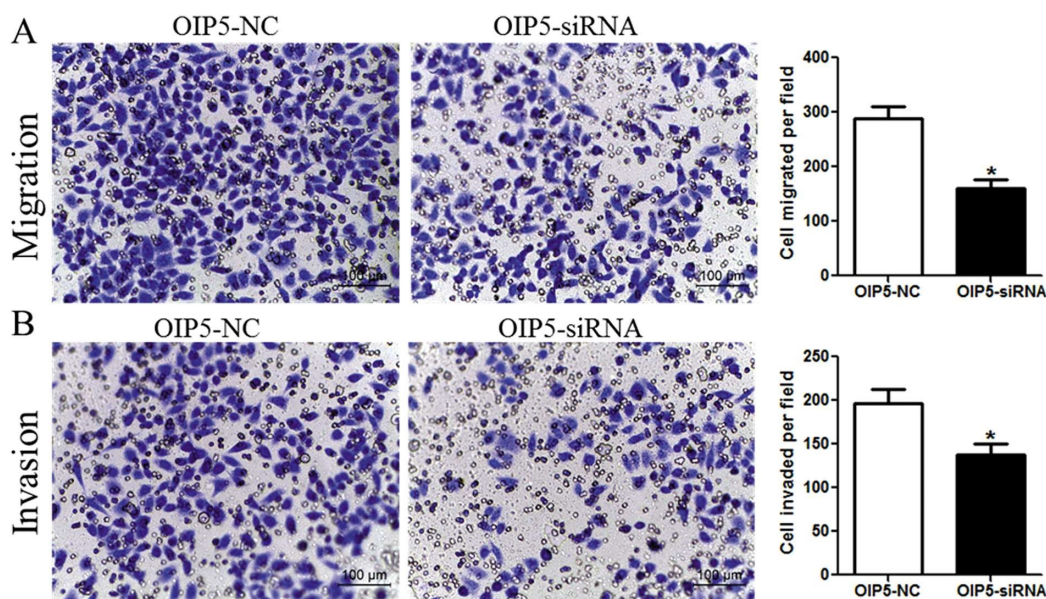


图3 转染 OIP5-siRNA 对 SMMC-7721 细胞侵袭迁移能力的影响

Fig.3 The effect of transfection with OIP5-siRNA on the cell migration and invasion ability of SMMC-7721 cells

3 讨论

由于肝癌的发生发展涉及多因素、多步骤和多种信号通路的复杂的过程^[10,11],肝癌患者的生存率仍然不佳,虽然其诊疗技术在不断进步,但大多数晚期肝癌患者手术切除的可能性很低,治疗方案有限,只能接受姑息性治疗^[12]。分子靶向药物的出现为晚期 HCC 患者带来福音,以索拉菲尼为代表的治疗 HCC 的分子靶向药物能够平均延长患者 2 到 3 个月的总体生存期^[13]。令人失望的是一系列新的肝癌靶向药物的临床研究显示舒尼替尼、布立尼布、利尼伐尼等药物均不能有效延长患者的生

存期^[14]。

CTA 能在多种肿瘤组织中表达,而在大多数正常组织不表达或少量表达,并且能在肿瘤患者体内引起体液和细胞免疫反应的特性,被认为是肿瘤靶向治疗的理想靶点。Kumar^[15]等研究发现 AKAP4 与卵巢癌的恶性特征相关,下调 AKAP4 后能够促进卵巢癌细胞发生凋亡,诱导周期抑制和抑制上皮间质化过程。Song^[16]等发现 NY-SAR-35 作为癌基因参与到肝癌和胰腺癌的增殖和侵袭迁移过程中。目前,MAGE-A1、MAGE-A3 和 NY-ESO-1 等 CTA 的一些成员的肿瘤应用治疗已进入到临床试验阶段,并在一些肿瘤的治疗中取得相当好的疗效^[17-19]。作为

CTA 家族成员之一, OIP5 的主要功能是参与调控细胞的有丝分裂过程, 对维持着丝粒的结构和功能起着重要的作用^[20,21]。研究表明 OIP5 在乳腺癌^[5,6]、肺癌^[7]、食管癌^[7]、胃癌^[8]、结肠癌^[8]和透明细胞癌^[9]等多种肿瘤中高表达, 在透明细胞癌患者中 OIP5 蛋白高表达组患者的预后比低表达组患者的预后差^[9], 在肺癌和食管癌患者中 OIP5 蛋白阳性患者的预后同样比阴性患者预后差, 提示 OIP5 可能作为一个癌基因参与肿瘤发生发展的过程。因此, 本实验首先采用 siRNA 技术靶向沉默 OIP5 在肝癌细胞 SMMC-7721 中的表达, 然后通过 CCK-8、平板克隆和 Transwell 实验探讨 OIP5 对肝癌细胞增殖、侵袭和迁移能力的影响。

肿瘤细胞的增殖和侵袭能力影响着肿瘤的生长速度和转移能力, 进而影响肿瘤的发生发展^[22]。浸润和转移能力是肿瘤的一大特征, 肝癌患者发生转移是肝癌治疗失败和患者死亡的主要原因^[23,24], 并与肝癌患者的预后息息相关^[25-27]。既往的研究表明 OIP5 与肿瘤的增殖能力息息相关, 在乳腺癌^[5,6]、肺癌^[7]、食管癌^[7]、胃癌^[8]、结肠癌^[8]和透明细胞癌^[9]等肿瘤中下调 OIP5 的表达后能够抑制肿瘤细胞的增殖能力, 但其对肝癌细胞侵袭迁移能力的影响尚未见报道。本研究首先采用 CCK-8、平板克隆检测 OIP5 对肝癌细胞增殖能力的影响, 发现下调 OIP5 表达的肝癌细胞增殖能力下降; 随后采用 Transwell 实验检测 OIP5 对肝癌细胞侵袭迁移能力的影响, 发现下调 OIP5 表达的肝癌细胞侵袭迁移能力明显低于对照组, 表明 OIP5 的表达水平影响着肝癌细胞的侵袭迁移能力。Li^[28]等新近研究发现 OIP5 通过调控 mTORC1 and β -catenin 信号通路影响肝癌的增殖和侵袭能力, 下调 OIP5 后肝癌细胞的增殖、侵袭和迁移能力明显降低。本实验的研究结果与 Li^[28]等新近研究结果一致, 共同表明下调 OIP5 后可以抑制肝癌的增殖、侵袭和迁移能力, 进而抑制肝癌的发生发展, 可能成为肝癌治疗的一个新靶标。

综上所述, 沉默 OIP5 的表达可抑制肝癌细胞的增殖、侵袭和迁移能力。后续研究中, 我们将在临床样本中探讨 OIP5 的表达, 并深入研究 OIP5 对肝癌增殖、侵袭和迁移能力影响的可能分子机制, 希望能够为肝癌的治疗提供新的依据。

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