HIP1基因沉默对人前列腺癌PC-3细胞增殖的影响

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【摘要】目的 研究HIP1基因沉默对人前列腺癌PC-3细胞增殖的影响。方法 构建靶向HIP1的shRNA表达载体pSilence-shH1P1，应用脂质体转染至PC-3细胞，RT-PCR检测HIP1基因沉默效果，Western blotting确认有效作用靶点。结果 通过细胞划痕实验和细胞生长曲线研究HIP1基因对PC-3细胞增殖的影响，转染PC-3细胞后，沉默HIP1基因效率达到83%（p<0.01）；Western blotting证实该基因片段为抑制HIP1的有效作用靶点。细胞划痕实验和生长曲线显示HIP1基因沉默可抑制PC-3细胞的增殖。

【关键词】HIP1基因；RNA干扰；PC-3细胞；细胞增殖

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Effect of HIP1 gene silencing on proliferation of PC-3 cells in human androgen-independent prostate tumor

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【Abstract】Objective To investigate the effect of Huntingtin-interacting protein1 (HIP1) gene silencing on the proliferation of PC-3 cells in a human androgen-independent prostate tumor. Methods pSilence-shHIP1, a shRNA expression vector targeting HIP1, was constructed and transfected into PC-3 cells by liposome. RT-PCR was adopted to detect the silencing effect of HIP1 gene. Western blotting was then used to confirm the effective target point. Moreover, the scratch assay and growth curve assay were performed to analyze the effect of HIP1 on the proliferation of PC-3 cells. Results The efficiency of HIP1 gene silencing was increased to 83% (p<0.01) after PC-3 cell transfection. Western blotting proved that this genetic fragment was the effective target point for the inhibition of HIP1. The scratch assay and growth curve assay showed that the proliferation of PC-3 cells was inhibited by silencing HIP1 gene. Conclusions The pSilence-HIP1 expression vector can specifically suppress the expression of the HIP1 gene. In addition, HIP1 gene silencing can inhibit the proliferation and migration of PC-3 cells.

【Key words】HIP1 protein; RNA interference; PC-3 cells; cell proliferation

近年来我国前列腺癌的发病率明显上升，许多老年患者发现时已属中晚期。随着对前列腺癌研究的深入，对前列腺癌的发病机制有新的认识。Huntingtin-interacting protein1 (HIP1)是一种具有多个结构域的蛋白质，由116个氨基酸组成。研究Huntingtin-interacting protein1与Huntingtin蛋白相互作用，可参与肿瘤的发生发展过程。有研究表明HIP1对于前列腺癌和结肠癌细胞的存活具有重要作用[1-4]，其在肿瘤进展中的作用尚不清楚。为此本研究构建了沉默HIP1的转染质粒，通过检测其对前列腺癌PC-3细胞增殖和迁移的影响，为进一步探讨HIP1在肿瘤发生发展中的作用。

1 材料与方法

1.1 实验材料 质粒pSILENCER(吉林大学白求恩医学院病理生理实验室惠赠)，大肠埃希菌(E. coli) DH5α（日本实验菌株保种），人前列腺癌PC-3细胞(上海中科院细胞所)，Trizol、脂质体Lipofectamine™ 2000(Invitrogen)，DNA 凝胶回收试剂盒(Omega)，RT-PCR试剂盒，限制性内切酶、DL2000(Takara)，胎牛血清、MEM培养基(Gibco)，T4DNA连接酶、反转录试剂盒(Promega)，质粒小提试剂盒(Tiangen)，细胞裂解液(Roche)，HIP1抗小鼠抗体、β-actin抗小鼠抗体、二抗(山羊抗小鼠抗体，Santa Cruz)，引物合成与测序由上海生工生物技术有限公司完成，其他试剂均为进口或国产分析纯。
1.2 方法
1.2.1 si-HIP1载体的构建 根据在线设计软件设计HIP1基因的RNA寡核苷酸序列。HIP1-1: GCACAGACGCATCTGATA(无效); HIP1-2: GACGGCATTATGGACCGA(无效); HIP1-3: GACCGAGGATGACTAC(有效); 阳性对照序列: TTTTCGAAAGTGGTCACGT。上述序列由上海生工生物技术有限公司合成。95°C 5min退火制备双链DNA oligo，经1:100稀释后连接到BamH I和Hind III双酶切的线性化pSILENCER载体上，连接产物分别转化大肠埃希菌感受态DH5α，挑取克隆进行PCR及酶切鉴定，阳性克隆进行测序鉴定，分别命名为Scramble和si-HIP1。

1.2.2 细胞培养及转染细胞株的获得 用含10%胎牛血清的IMEM培养液培养PC-3细胞。采用脂质体Lipofectamine®tm 2000，将对照质粒Scramble和si-HIP1分别转染至PC-3细胞，48h后收集细胞进行下一步实验。

1.2.3 RT-PCR检测细胞中HIP1 mRNA水平 收集转染si-HIP1及对照质粒Scramble 48h后的细胞，用Trizol提取总RNA，各取5μg反转录成cDNA。－20°C保存。HIP1引物序列：上游5′- CAAGTGATTTTGGAACGAG-3′; 下游5′-AGCAGGTTATGGATGGA-3′，扩增片段长度为595bp。PCR反应体系：上、下游引物各0.5μl, 2×PCR缓冲液10μl, cDNA1μl, 双蒸水8μl，总体积为20μl。PCR反应条件：94°C 5min; 94°C 1min, 55°C 30s, 72°C 1min, 30个循环; 72°C延伸10min。采用上海天能2500R型凝胶成像系统采集图像。Quantity One软件定量分析。

1.2.4 Western blotting检测HIP1蛋白的表达 收集si-HIP1及对照质粒Scramble转染48h后的细胞，提取细胞总蛋白，定量，取60μg蛋白进行SDS-PAGE电泳(SDS-PAGE胶浓度为12%), 电转至PVDF膜，5%脱脂奶粉室温封闭1h，分别加入1:200稀释的鼠抗HIP1一抗和鼠抗B-actin一抗，4°C孵育过夜后，用TBST洗3次，再分别加入1:1000稀释的辣根过氧化物酶标记的羊抗鼠二抗，室温孵育1h，再用TBST洗3次。DAB显色，上海天能2500R型凝胶成像系统采集图像，Quantity One软件定量分析。
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Fig. 2 Assessment of HIP1 gene silencing by RT-PCR
1. Scramble; 2. si-HIP1-1; 3. si-HIP1-2; 4. si-HIP1-3; (1) *P* < 0.01 compared with Scramble group

Fig. 3 Detection of protein expression after HIP1 gene silencing by Western blotting
1. Scramble group; 2. si-HIP1-3 group; (1) *P* < 0.01 compared with scramble group

Fig. 4 The effect of HIP1 gene silencing on the cell proliferation and migration of PC-3 cells

*Fig. 5* The effect of HIP1 gene silencing on the cell growth rate of PC-3 cells

*P* < 0.05, (1)

**Discussion**

Recent research has shown that HIP1 can be regulated by different signal pathways, leading to the development and progression of certain cell processes. HIP1 is overexpressed in various types of tumors, such as breast cancer, ovarian cancer, prostate cancer, and rectal cancer. This overexpression may contribute to the progression of these tumors due to its role in regulating cell proliferation and migration. By silencing HIP1, we can observe the effects on cell proliferation and migration, as shown in Figs. 3 and 4.

Furthermore, the effect of HIP1 gene silencing on the growth rate of PC-3 cells is demonstrated in Fig. 5. The results show a significant decrease in cell count compared to the scramble group, highlighting the critical role of HIP1 in cell proliferation and growth.

Discussion

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细胞HIP1基因表达后，细胞的增殖和迁移速度均明显减慢，提示HIP1基因可能参与了细胞增殖和迁移的调控。

RNA干扰是一种常用的基因转录后沉默技术，通常利用带有抗性基因或荧光质粒的载体来构建表达小RNA的干扰质粒，进而通过抗性或荧光筛选得到稳定转染的细胞株，并在此基础上进行目的基因的功能作用研究。本研究采用带有抗性基因的载体，通过基因重组构建HIP1的基因干扰表达载体，通过Lipofectamine™ 2000转染PC-3细胞，分别经RT-PCR和免疫印迹实验，从mRNA和蛋白水平进行验证，获得了干扰HIP1表达的有效靶点。为研究HIP1基因在细胞增殖和迁移方面的作用提供了实验基础。试验进一步实验和生长曲线显示HIP1基因被干扰后PC-3细胞的迁移、分裂和增殖均显著受抑，推测HIP1基因可能与PC-3细胞的周期调控有关。

综上所述，本研究成功地在PC-3细胞中沉默了HIP1基因，为进一步研究其在前列腺癌中的作用及具体机制奠定了基础。

【参考文献】

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