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KLF4对肿瘤干细胞自我更新和增殖潜能的影响

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摘要：

目的： 分析Krüppel样因子4（Krüppel-like factor 4，KLF4）对肿瘤干细胞 T3A-A3自我更新和增殖能力的影响。 方法： 构建靶向干扰 KLF4 的慢病毒载体pLVTHM-shKLF4，利用前期实验分离与鉴定的肿瘤干细胞T3A-A3，应用RT-PCR和Western blotting分别检测pLVTHM-shKLF4感染后T3A-A3细胞中 KLF4 mRNA和蛋白的表达。细胞球形成实验检测pLVTHM-shKLF4感染对T3A-A3细胞自我更新的影响，平板集落形成实验检测T3A-A3细胞的克隆形成能力，流式细胞术检测T3A-A3细胞周期变化。裸鼠皮下移植瘤实验观察pLVTHM-shKLF4干扰 KLF4 表达后对T3A-A3细胞移植瘤生长的影响。 结果： 与肝癌细胞BEL-7402、HepG2相比，肿瘤干细胞T3A-A3表达更高水平的 KLF4 ；pLVTHM-shKLF4感染能够在mRNA和蛋白水平下调T3A-A3细胞中 KLF4 的表达。pLVTHM-shKLF4感染的T3A-A3细胞形成细胞球的直径明显小于对照病毒的pLVTHM-shNC感染的T3A-A3细胞\[（ 104.33±16.28） vs (186.67±28.15) μm， P <0.01\]，pLVTHM-shKLF4感染细胞形成的细胞克隆数目明显少于对照细胞\[（83.5±7.78） vs (125±9.19)个， P <0.01），pLVTHM-shKLF4感染的T3A-A3细胞G 1期比例明显升高\[（39.65±4.03）% vs (29 35±1.00)%， P <0.01\]。pLVTHM-shKLF4感染的T3A-A3细胞的移植瘤生长速度较对照细胞移植瘤明显减慢\[细胞接种33 d，(46.14±12.94) vs (228.12±94.86) mm 3， P <0.01\]。 结论： 干扰 KLF4 的表达可抑制肿瘤干细胞T3A-A3的自我更新及其在体内外的增殖潜能。

关键词：Krüppel样因子4（KLF4） RNA干扰 肝癌 肿瘤干细胞 T3A-A3 自我更新 增殖

Effect of KLF4 on self-renewal and proliferation potential of tumor stem cells

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Abstract:

Objective: To explore the effect of Krüppel-like factors 4 (KLF4) on self-renewal and proliferation potential of tumor stem cells （T3A-A3）. Methods: A lentiviral vector carrying shRNA targeting KLF4 (pLVTHM-shKLF4) was constructed. Tumor stem cells (T3A-A3 cells) were isolated from a human hepatocarcinoma and were identified in our previous study. The expression of KLF4 mRNA and protein in T3A-A3 cells was analyzed by RT-PCR and Western blotting analysis after pLVTHM-shKLF4 infection. Self-renewal ability of T3A-A3 cells was evaluated by tumor sphere formation assay after pLVTHM-shKLF4 infection; clonogenic assay was used to determine the clonogenic ability of T3A-A3 cells; and cell cycle phase distribution was analyzed by flow cytometry. Influence of KLF4 knockdown on the growth of T3A-A3-transplanted tumors was examined in xenograft model of nude mice. Results: T3A-A3 expressed higher level of KLF4 than human hepatocarcinoma cell line Bel-7402 and HepG2. pLVTHM-shKLF4 infection significantly decreased the expression of KLF4 mRNA and protein in T3A-A3 cells. The formed tumor spheres of T3A-A3 cells were significantly smaller in pLVTHM-shKLF4 infection group compared with that in the pLVTHM-shNC control group (\[104.33±16.28\] μm vs \[186.67±28.15\] μm, P <0.01). pLVTHM-shKLF4 infection significantly inhibited the number of T3A-A3 cell-colonies compared with control group (83.5±7.78 vs 125±9.19, P <0.01). Flow cytometry analysis showed that pLVTHM-shKLF4 infection significantly increased G 1 population when compared with the control vector (\[39.65±4 03\]% vs \[29.35±1.00\]%, P <0.01). Furthermore, the growth of T3A-A3-transplanted tumors in pLVTHM-shKLF4 infection group was significantly slower than that in the control group (33 days after cell inoculation, \[46.14±12 94\] vs \[228.12±94.86\] mm 3, P <0.01). Conclusion: KLF4 knockdown can inhibit the self-renewal of tumor stem cells (T3A-A3 cells), and inhibit the proliferation potential of T3A-A3 both in vitro and in vivo .

Keywords:Krüppel-like factor 4 (KLF4) RNA interference hepatocellular carcinoma tumor stem cell T3A-A3 self renewal proliferation

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