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Survivin-pPRIME-IGF1R-miR30慢病毒载体的构建及其对肝癌细胞生长的抑制

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牛 坚 刘 斌 于 彬 王 月 李向农

徐州医学院附属医院 普外科，江苏 徐州 221004;徐州医学院附属医院 普外科，江苏 徐州 221004;徐州医学院附属医院 普外科，江苏 徐州 221004;徐州医学院附属医院 普外科，江苏 徐州 221004;徐州医学院附属医院 普外科，江苏 徐州 221004

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摘要：

目的：探讨survivin启动子调控的重组慢病毒survivin pPRIME IGF1R miR30载体(简写为sur IGF1R miR30)对肝癌Hep3B细胞IGF1R表达和细胞生长的影响。方法：PCR扩增survivin启动子，构建sur pPRIME；将针对 〖STBX〗IGF1R 〖STBZ〗基因的干扰序列与sur pPRIME载体连接，构建sur IGF1R miR30慢病毒载体。将sur IGF1R miR30、psPAX2和pMD2G质粒共转染293T细胞，扩增慢病毒，检测病毒滴度。以sur IGF1R miR30感染人肝癌Hep3B细胞和胎肝L 02细胞，RT PCR、Western blotting检测Hep3B细胞IGF1R的表达，CCK8法检测Hep3B细胞的生长。结果：成功构建survivin启动子调控的慢病毒载体sur IGF1R miR30，滴度为4.58×10 9 PFU/ml。Sur IGF1R miR30感染后，Hep3B细胞中特异表达荧光蛋白，L 02细胞中基本没有表达。Sur IGF1R miR30感染Hep3B细胞可阻断 IGF1R mRNA和IGF1R蛋白的表达。Sur IGF1R miR30感染抑制肝癌细胞的生长，第7天时的抑制率达60%(P < 0.05)。结论：成功构建的重组慢病毒载体sur IGF1R miR30 可有效地降低肝癌细胞中IGF1R的表达，抑制肝癌细胞的增殖。

关键词：肝肿瘤 人胰岛素样生长因子1类受体 survivin启动子 慢病毒 RNA干扰

Construction of survivin pPRIME IGF1R miR30 lentiviral vector and its inhibitory effect on proliferation of liver cancer cells

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NIU Jian LIU Bin YU Bin WANG Yue LI Xiang-nong

Department of General Surgery, Affiliated Hospital of Xuzhou Medical College, Xuzhou 221004, Jiangsu, China;Department of General Surgery, Affiliated Hospital of Xuzhou Medical College, Xuzhou 221004, Jiangsu, China;Department of General Surgery, Affiliated Hospital of Xuzhou Medical College, Xuzhou 221004, Jiangsu, China;Department of General Surgery, Affiliated Hospital of Xuzhou Medical College, Xuzhou 221004, Jiangsu, China;Department of General Surgery, Affiliated Hospital of Xuzhou Medical College, Xuzhou 221004, Jiangsu, China

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Abstract:

Objective:To study the effects of survivin promoter regulated survivin IGF1R miR30 (sur IGFIR miR30) lentiviral vector on the IGF1R expression and proliferation of hepatoma Hep3B cells. Methods：Survivin promoter was amplified by PCR and sur pPRIME plasmid was constructed. Interference sequence targeting IGF1R gene was synthesized and cloned into sur pPRIME plasmid, named sur IGF1R miR30. Sur IGF1R miR30, psPAX2, and pMD2G were co transfected into 293T cells to amplify lentivirus, and the lentivirus titer was examined. IGF1R expression in Hep3B cells was detected by RT PCR and Western blotting analysis, and the proliferation of Hep3B cells was evaluated by CCK 8 assay. Results：Sur IGF1R miR30 lentiviral vector regulated by survivin promoter were successfully constructed, and the virus titer was 4.58×10 9 PFU/ml. Fluorescent protein after sur IGF1R miR30 infection was expressed in Hep3B cells, but not in L 02 cells. Sur IGF1R miR30 infection inhibited IGF1R mRNA and protein expressions in Hep3B cells and the proliferation of Hep3B cells, with the inhibitory rate being 60% at 7 d (P <0.05). Conclusion: Sur IGF1R miR30 lentiviral vector can inhibit IGF1R expression and hepatoma cell proliferation.

Keywords:liver neoplasms human insulin like growth factor receptor 1 survivin promoter lentivirus RNA interfere

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