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论著

携带DREAM基因shRNA的慢病毒载体的构建及其对坐骨神经缩窄损伤大鼠的镇痛作用

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摘要
目的：构建含下游调控元件的拮抗分子（downstream regulatory element antagonistic modulator，DREAM）基因有效短发夹RNA(shRNA)的慢病毒载体, 利用RNA干扰技术抑制DREAM基因表达，开展对神经病理性疼痛基因治疗的实验研究。方法：将靶向大鼠DREAM基因的shRNA重组到慢病毒穿梭质粒中，将重组质粒pKCSHR-puro/GFP-DREAM和慢病毒包装系统共转染293T细胞包装成慢病毒，收集病毒上清并进行载体病毒滴度的测定。将纯种健康清洁级成年雄性SD大鼠36只随机分为6组:正常对照组(N)、假手术组(S)，根据干预手段的不同将大鼠坐骨神经缩窄损伤（chronic constriction injury of sciatic nerve，CCI）模型组分为4组:CCI(C0)组，CCI疼痛模型建立后不给任何干预措施,生理盐水对照（C1）组，空白载体对照（C2）组，LV-shRNADREAM慢病毒载体治疗（C3）组。该3组大鼠于CCI后第7天分别于蛛网膜下腔注射生理盐水、空白载体、LV-shRNADREAM慢病毒载体。观察各组大鼠术后3,7,10,14,21 d的疼痛行为学的改变。实验结束取腰段脊髓荧光显微镜检测绿色荧光蛋白(GFP)的表达，Realtime-PCR测定DREAM基因的mRNA 含量、Western印迹测定DREAM 蛋白含量。结果：测序分析证实载体构建成功，成功进行慢病毒包装，得到病毒液滴度为1.0×108 IFU/mL慢病毒载体。鞘内注射LV-shRNADREAM慢病毒载体后, CCI大鼠热痛阈和机械痛阈的异常明显得到改善（P<0.01）, 腰段脊髓神经细胞DREAM基因mRNA和 DREAM蛋白的表达均显著降低（P<0.01）。结论：成功构建了携带大鼠DREAM基因有效shRNA的慢病毒载体，慢病毒携带的短发夹干扰ＲＮＡ能干扰脊髓中DREAM的表达，为神经病理性疼痛的治疗提供了实验基础。

关键词 RNA干扰 神经病理性疼痛 下游调控元件拮抗分子 慢病毒载体

分类号

扩展功能

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Construction of shRNA lentivirus vector on rat DREAM gene and its analgesic effect on CCI rats

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Abstract
ObjectiveTo construct the recombinant lentivirus vector containing short hairpin RNA (shRNA) inhibited DREAM expression and to investigate the gene therapy of neuropathic pain by inhibiting the expression of DREAM gene by RNA interference. MethodsAn effective short hairpin RNA targeting to rat DREAM was cloned into the plasmids on the base of Lentivirous vectrs, pKCSHR-Puro/GFP，and both of the pKCSHR-Puro/GFP-DREAM and Lentivector package plasmids mix were transferred into the 293T cells. The culture supernatant was harvested, and the virus titer was detected 48 hours after transferring. Thirty-six sheer breed pathogen free adult Sprague Dawley rats were randomly divided into 6 groups(6 in each group): normal control group (N); sham-operated group (S); CCI group (C0 group):CCI model without any intervention; Saline control group (C1 group); empty vector control group (C2 group); and LV-shRNADREAM lentiviral vector treatment group (C3 group). The rats in the last 3 groups respectively accepted injection of normal saline, blank vector, LV-shRNADREAM lentiviral vector in the subarachnoid on the 7th day after CCI, and the pain behavior was observed after 3,7,10,14,21 d after CCI.Green fluorescent protein (GFP) expression was detected by fluorescence microscope and the contents of DREAM mRNA and DREAM protein were detected by Realtime PCR and Western blot respectively in the rat lumbar spinal cord.ResultsThe short hairpin RNA sequences targeting at rat DREAM were cloned into the vectors, and an entry clone and an expression clone were constructed successfully confirmed by sequence analysis. Lentiviral packaging was successful in 293 T cell line and the transfection titer of the lentivirus was 1×108 IFU/mL. LV-shRNADREAM lentivirus vector was transfected successfully in the rat spine with Intrathecal injection of LV-shRNADREAM. Compared with the other 3 groups, heat

pain threshold and mechanical pain threshold in Group C3 improved significantly （P<0.01）, and the expression of DREAM mRNA and DREAM protein in the lumbar spinal cord in Group C3 were lowered significantly(P<0.01).ConclusionLentivirus vectors containing rat DREAM gene are constructed successfully, and lentivirus mediated shRNA can inhibit the DREAM expression in the rat spine, which may prove to be an effective method for neuropathic pain.

Key words RNA interference neuropathic pain DREAM Lentivirus vector

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