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双链腺相关病毒介导表达Exendin-4治疗糖尿病大鼠分享到:

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Title: Efficiency of Exendin-4 expression mediated by a recombinant double-stranded adeno-associated virus vector in treatment of diabetic rats

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关键词: [双链AAV](#); [Exendin-4](#); [2型糖尿病大鼠](#)

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摘要: 目的 构建、鉴定自身互补双链DNA的腺相关病毒(scAAV)重组载体,使其分泌表达Exendin-4,检测其转导效率并观察在糖尿病大鼠模型中的治疗作用。 方法 应用基因工程方法改建穿梭质粒pSSHG-CMV,插入外源性基因Exendin-4,构建重组scAAV载体,感染HEK293细胞,ELISA检测转染NIH3T3细胞上清Exendin-4滴度,链佐霉素诱导20只6周龄体质量180~220 g SD成年雄性大鼠为糖尿病鼠模型,逆向注射重组scAAV于糖尿病大鼠颌下腺,检测其血糖及胰岛素分泌水平。 结果 重组scSSHG/exn4可有效包装和复制,病毒滴度为

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2.5×10^{11} pfu/mL, 转染细胞上清分泌Exendin-4浓度可达到4.53 ng/mL, scAAV治疗组血糖浓度在2、4周及8周均低于对照组[分别为 (639.17 ± 27.89) vs (396.00 ± 34.00) , (657.02 ± 39.87) vs (315.62 ± 42.56) , (215.6 ± 24.7) vs (458.6 ± 19.7) mg/dL], 胰岛素浓度均高于对照组[分别为 (156.8 ± 24.5) vs (535.9 ± 35.6) , (236.5 ± 12.3) vs (495.3 ± 18.6) , (620.43 ± 46.90) vs (381.56 ± 21.78) pg/mL], 二者比较有显著统计学差异($P < 0.05$)。 结论 成功构建重组双链腺伴病毒scAAV-Ex-4, 具有高效转导能力, 对糖尿病大鼠模型具有控制血糖及增加胰岛素分泌作用。

Abstract: **Objective** To construct and identify a self complementary double-stranded recombinant adeno-associated virus vector (scAAV) to secrete Exendin-4 (Ex-4). The transduction efficiency of the recombinant scAAV-Ex-4 was evaluated and its efficacy in treatment of diabetic rats was observed. **Methods** The shuttle vector pSSHG-CMV inserted with Ex-4 gene was constructed by genetic engineering method. The scAAV-Ex-4 vector was co-transferred into HEK-293 cells. Ex-4 level in the supernatants from transfected NIH3T3 cells was determined by an enzyme immunoassay kit. The rat model of type 2 diabetes (T2DM) was successfully induced by streptozotocin in twenty six-week-old Sprague-Dawley (SD) male rats (160-180 g). The recombinant scAAV was injected into the salivary gland of T2DM model rats. The levels of blood glucose and insulin of the rats were assessed. **Results** The recombinant scAAV-Ex-4 was successfully constructed and packaged with a high titer of virus (2.5×10^{11} pfu/L). The Ex-4 concentration of the supernatants from