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论著

多巴胺D1受体RNA干扰载体的构建及其沉默效应鉴定

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摘要： 目的: 构建多巴胺D1受体(DRD1)表达干扰载体,为研究DRD1在神经细胞中的作用及抗惊厥药物打下基础。方法: 根据GenBank中DRD1基因序列,设计10个干扰序列并构建DRD1干扰载体。对NG-108-15进行脂质体转染后,GFP标记检测转染效果。Real-time PCR和Western印迹检测NG-108-15中DRD1的表达量。结果: 构建的10个干扰载体均能采用脂质体法转染到NG-108-15细胞中。转染后NG-108-15中DRD1 mRNA和蛋白的表达均明显下降。其中转染pGPU6-GFP-Neo-si-DRD1-5载体的NG-108-15中DRD1 mRNA表达水平最低,而转染pGPU6-GFP-Neo-si-DRD1-1,-2,-6,-7载体的NG-108-15中DRD1 蛋白表达水平最低。结论: 成功构建了DRD1表达干扰载体。

关键词： 多巴胺D1受体 RNA干扰 NG-108-15细胞

Construction of RNAi vector of dopamine D1 receptor and identification of its silencing effects

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Abstract: Objective: To construct dopamine D1 receptor (DRD1) expression interference vectors to study the role of DRD1 in nerve cells and lay a foundation for drug development in anti-convulsion.
Methods: Based on DRD1 gene sequence in GenBank, 10 interfere vectors of DRD1 were designed. Liposomal was used to transfect NG-108-15 and the transfect effect was assayed by GFP. With realtime PCR and Western blot, the DRD1 expression was detected.
Results: The 10 constructed interfere vectors transfected into NG-108-15 cells by liposomal method and inhibited DRD1 mRNA and protein expression. DRD1 mRNA expression in NG-108-15 cells transfected with pGPU6-GFP-Neo-si-DRD1-5 was the lowest whereas DRD1 protein expression in NG-108-15 cells transfected with pGPU6-GFP-Neo-si-DRD1-1, -2, -6, -7 was the lowest.
Conclusion: DRD1 expression interference vector is successfully constructed.

Keywords: dopamine D1 receptor RNA interference NG-108-15 cell

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