学报简介

第二军医大学学报 ISSN 0258-879X CN 31-1001/R

ACADEMIC JOURNAL OF SECOND MILITARY MEDICAL UNIVERSITY

卞金俊1，朱科明1，万小健1，邓小明1*，黄盛东2，袁扬2，龚德军2. 体外筛选针对大鼠Toll样受体4 mRNA的小分子干扰RNA序列[J]. 第二军医大学学报, 2008, 29(1): 0048-0052

体外筛选针对大鼠Toll样受体4 mRNA的小分子干扰RNA序列

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基金项目：

DOI：10.3724/SP.J.1008.2008.00048

摘要：

目的：筛选能高效干扰大鼠Toll样受体4（Toll-like receptor 4，TLR4）mRNA的最佳小分子干扰RNA（small interfering RNA, siRNA）序列。方法：克隆大鼠TLR4基因全长，将TLR4基因与含增强型绿色荧光蛋白（enhanced green fluorescent protein, EGFP）的质粒pEGFP-C1重组，构建pEGFP-rTLR4，化学合成法合成3对干扰大鼠TLR4的siRNA后，将3对siRNA、阴性对照siRNA和干扰EGFP的siRNA分别与pEGFP-rTLR4经Lipofectamine2000共转染HEK-293细胞株，通过倒置相差显微镜和流式细胞仪观察EGFP的荧光强度。结果：与阴性对照组相比，3对针对TLR4的siRNA及针对EGFP的siRNA均明显抑制EGFP的荧光表达（$P<0.05$）。其中尤以siRNA2（核苷酸序列为5′-GTC TCA GAT ATC TAG ATC T-3′，位于TLR4基因序列的1 352～1 370位）的抑制效果最强，干扰效率>75%。结论：成功筛选出体外可高效干扰大鼠TLR4 mRNA的siRNA片段。

关键词：小分子干扰RNA Toll样受体4 PCR 化学合成法

Screening for siRNA sequence targeting rat Toll-like receptor 4 mRNA in vitro

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Abstract:

Objective: To screen for an optimized siRNA sequence targeting rat Toll-like receptor 4 (TLR4) in vitro. Methods: The full length gene of rat TLR4 was cloned and inserted into pEGFP-C1 plasmid to construct pEGFP-rTLR4. Three pairs of siRNAs targeting rTLR4 were chemically synthesized and were co-transfected with pEGFP-rTLR4 into HEK-293 cells via Lipofectamine2000. Cells were also co-transfected with siRNA targeting EGFP and negative control siRNA. The expression of EGFP was observed under inverted fluorescene microscope and flow cytometry. Results: Compared with the negative control group, 3 pairs of siRNAs targeting TLR4 and one pair of siRNA targeting EGFP significantly suppressed the EGFP expression ($P<0.05$); the inhibitory effect of siRNA2 (gene sequence: 5′-GTC TCA GAT ATC TAG ATC T-3′, 19 bp, 1 352-1 370) was the strongest one, with an interference efficiency over 75%. Conclusion: We have successfully obtained the siRNA sequence targeting TLR4 mRNA, which can efficiently suppress the expression of rat TLR4 mRNA in vitro.

Keywords: small interfering RNA Toll like receptor 4 PCR chemical synthesis

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