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论著

人抗肌萎缩蛋白Dp71 shRNA载体构建与检测

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摘要：

目的：构建有效针对人Dystrophin Dp71基因的短发夹RNA(short hairpin RNA，shRNA)真核表达载体，并验证其干扰效果。方法：设计合成3对针对人Dystrophin Dp71基因的和1对无同源性的siRNA片段，在两端和中间加上酶
切位点和Loop环。将合成的DNA片段插入到干扰载体pRNAT-U6.1/Neo中，经过酶切和测序验证，成功构建人Dp71基
因的shRNA和空白对照载体。将各干扰载体和空白载体转染人正常胃黏膜上皮细胞(gastric epithelial cells，GES-1)和人
支气管上皮细胞(human bronchial epithelium，HBE)，Western印迹检测Dp71 shRNA真核表达载体的干扰效率。结果：酶
切和测序验证均表明各Dp71-shRNA载体构建成功。将空载体及各Dp71 shRNA载体分别转染GES-1和HBEC，和空白细
胞对照及shRNA空白载体组相比，3组Dp71-shRNA能够明显抑制Dp71蛋白表达，但是3组质粒的抑制效率有一定的差
异，以Dp71 shRNA2对Dp71表达的干扰效率最强。结论：成功构建了3个有效针对人Dystrophin Dp71基因的shRNA 干
扰载体，3组质粒都能有效地抑制Dp71在GES-1和HBEC中的表达，其中以Dp71-shRNA2的抑制效率最高。

关键词： Dystrophin Dp71 shRNA 载体 干扰效率

Construction and evaluation of human Dp71 shRNA vector

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Abstract:

Objective: To construct effective short hairpin RNA (shRNA) recombinant plasmids targeting human Dystrophin Dp71 gene, and evaluate their interference efficiency.
Methods: Three pairs of siRNA sequences targeting human Dp71 gene and one pair of control siRNA sequence were designed, synthesized, and then inserted into the pRNAT-U6.1/Neo vector. The shRNA recombinant vectors were evaluated by enzyme digestion and sequencing. Dp71-shRNA and control shRNA plasmids were transfected into human normal gastric epithelial cells (GES-1) and human bronchial epithelium (HBE). Western blot was used to evaluate its interfering efficiency.
Results: Restriction enzyme digestion and sequencing showed that the Dp71-shRNA vectors were successfully constructed. Western blot displayed that Dp71 protein expression was reduced to a significant degree after transfection with the 3 Dp71-shRNA plasmids, and Dp71-shRNA2 plasmid inhibit the Dp71 expression most efficiently.
Conclusion: Dp71-shRNA vectors have been successfully constructed. The 3 Dp71-shRNA plasmids can inhibit Dp71 expression in GES-1 and HBEC, with Dp71-shRNA2 plasmid displaying the highest inhibition efficiency.

Keywords: Dystrophin Dp71 shRNA plasmid inhibition efficiency

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