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[1]刘淑宝,田衍平,陈显军,等.DISC1基因过表达对星形胶质细胞质膜伸展的影响[J].第三军医大学学报,2013,35(10):938-942.

Liu Shubao, Tian Yanping, Chen Xianjun, et al. Effect of DISC1 overexpression on membrane expansion of astrocytes[J]. J Third Mil Med Univ, 2013, 35(10):938-942.

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## DISC1基因过表达对星形胶质细胞质膜伸展的影响。

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Title: Effect of DISC1 overexpression on membrane expansion of astrocytes

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关键词: 精神分裂症断裂基因1; 真核表达质粒; 星形胶质细胞; 质膜伸展

Keywords: disrupted-in-schizophrenia 1; eukaryotic expression plasmid; astrocytes;

membrane expansion

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摘要: 目的 构建精神分裂症断裂基因1 (Disrupted-In-Schizophrenia 1, DISC1) 蛋白表

达质粒, 检测其过表达后对星形胶质细胞 (Astrocytes, ASTs) 质膜伸展的影响。

方法 针对小鼠DISC1基因mRNA编码序列,设计合成可扩增全长引物;以小鼠脑白质 cDNA为模板进行PCR扩增,构建全长表达质粒,命名为pEGFP-n1-DISC1。分别将过表 达组pEGFP-n1-DISC1和空载体对照组pEGFP-n1电转染原代培养ASTs, 以Western blot 及免疫荧光染色检测DISC1-GFP融合蛋白的表达,利用Image-Pro Plus 5.0软件分析对照 组与DISC1过表达组星形胶质细胞质膜伸展面积的大小。 结果 成功从cDNA中扩增出DISC1编码序列并插入pEGFP-n1载体,双酶切和测序结果证实,DISC1蛋白编码序列插入载体,并能正确读码翻译。构建质粒转染ASTs 60 h后,可见DISC1-GFP融合蛋白表达:与对照组相比,DISC1过表达组ASTs的质膜伸展面积明显增加(P<0.05)。

结论 成功构建小鼠DISC1全长基因表达载体,DISC1基因过表达后可促使ASTs质膜伸

展面积增加。

Abstract: Objective To construct an expression plasmid vector carrying disrupted-in-

schizophrenia 1 (DISC1) gene, and to determine the effect of DISC1  $\,$ 

overexpression on membrane expansion of astrocytes. Methods Primers for the full-length of mouse DISC1 gene was designed and synthesized. The coding sequence of DISC1 gene was amplified by PCR from cDNA reversed from mouse

white matter RNA, and then was inserted into a vector pEGFP-n1 using

recombinant DNA technique. After verified by enzyme digestion and DNA

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sequencing, the recombinant plasmid named pEGFP-n1-DISC1 (DISC1 overexpression group) and the empty control pEGFP-n1 vector (control group) were transferred into astrocytes by electroporation, respectively. The expression of DISC1-GFP fusion protein in astrocytes was detected by Western blotting and immunofluorescent staining. The membrane expansion of astrocytes in the DISC1 overexpression group and control group were determined by analyzing the extended area of astrocytes with software Image-Pro Plus 5.0. Results The enzyme digestion and DNA sequencing confirmed that the coding sequence of mouse DISC1 was consistent with that in GenBank. The DISC1 was cloned into pEGFP-n1 vector in a right direction and named as pEGFP-n1-DISC1. The recombinant and control plasmids were effectively transferred into astrocytes with 40% of cells emitting green fluorescence after 60 h of transfection. The expression of DISC1 was only observed in cells transfected with pEGFP-n1-DISC1, and the membrane extended area of the DISC1 overexpression group significantly increased as compared with that of the control group (P<0.05).An exogenous expression plasmid vector carrying mouse DISC1 is successfully constructed, and DISC1 overexpression may induce the membrane expansion of astrocytes.

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