论著

酵母双杂交系统筛选CLN8P相互作用蛋白

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摘要 目的:应用酵母双杂交系统筛选CLN8P相互作用蛋白质,通过对相互作用蛋白质的筛选及研究,探讨CLN8的功能,为NCL8和NCLs疾病群的发病机制研究提供线索,并为疾病的蛋白质相互作用网络提供资料。方法 应用酵母双杂交技术,以pLexA-CLN8为诱饵质粒筛选人胎脑cDNA文库,得到Leu+LacZ+阳性克隆,进一步进行验证,并对验证后的阳性克隆的外源性片断进行测序及同源性分析。结果 从人胎脑cDNA文库中筛选得到60个Leu+LacZ+阳性克隆;将获得的具有外源性片段的文库质粒与诱饵质粒一对一重新转入酵母体内对相互作用进行验证,共获得阳性克隆22个;对验证后阳性克隆测序并进行同源性分析,共获得不同的候选基因序列10个。结论 应用酵母双杂交系统,共筛选得到10个不同的基因,其编码蛋白与CLN8P有相互作用,可能与NCLs发病机制相关。

关键词 <u>酵母双杂交技术</u> <u>神经元蜡样脂褐质沉积症</u> <u>CLN8</u> <u>蛋白质相互作用</u> 分类号

Screening Interactive Proteins of CLN8 by Yeast Two Hybrid Technique

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Abstract Objective The function of CLN8 and its role in the NCLs pathogenesis is not clear no w. Our aim is to screen and identify the interactive protein of CLN8P, provide clues for the studie s on CLN8 function and pathogenesis of NCLs. Methods The Human Fetal Brain cDNA library was screened with with pLexA-CLN8 as bait plasmid by yeast-two hybrid system, and Leu+ an d LacZ+ positive yeast clones were obtained, which were cotransformed to the EGY48 (p8op-L acZ) along with pLexA-CLN8 plasmid one to one by yeast simultaneous cotransformation so as to identify the protein interactions again. The inserted fragments of identified positive clones were sequenced and analyzed with blast in NCBI. Results There are 60 Leu+ and LacZ+ positive yeast clones which were obtained through screening the fetal brain library with pLexA-CLN8 as bait plasmid. There are 22 positive clones identified one to one by yeast simultaneous co-transformation. 10 different candidate gene sequences were obtained by blast analysis in NCBI. Conclusions 10 gene sequences were obtained, these coding proteins can interact with CLN8P, and some pf them may be involved with pathogenesis of NCLs.

Key wordsFONT-FAMILY: 'Times New Roman'mso-font-kerning: 1.0ptmso-ansi-language: EN-USmso-fareast-language: ZH-CNmso-bidi-language: AR-SAyeast two-hybrid syst

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