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小鼠小脑区域性特异表达基因的分离和筛选——乳化酚递减杂交[1)]

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摘要 哺乳类脑组织有着极其复杂和众多的基因表达，许多遗传性神经退行性疾病起源于不同的脑 功能区域的基因表达缺陷。分离和筛选小脑特异表达基因是认识小脑疾病的重要途径。本文 采用递减杂交法，大脑前皮层和肝组织单链cDNA为驱动物与小脑双链cDNA进行乳化酚杂交， 经过重组噬菌体λgt11 DNA连接选择，特异性地克隆那些仅在小脑中优势表达的cDNA。杂交 后的小脑cDNA文库容量仅是起始文库容量的0.049%(2.5×10 3/5/15×10 6)。用小脑和肝cD NA探针与PERT cDNA进行克隆杂交和斑点印迹杂交，结果显示绝大多数克隆仅与小脑cDNA杂 交阳性。选择10个克隆cDNA与小脑等5种组织mRNA做Northern杂交，有2个小脑特异表达克隆 ，6个小脑优势表达克隆。对其中PC7和PD8克隆cDNA做部分DNA序列分析，它们是以前未报道 过的新基因。

关键词 mRNA,cDNA,PCR,PERT杂交,小鼠小脑,cDNA文库,克隆的分离和筛选,分子杂交,DNA序 列分析

分类号

Screening and Isulation the Cerebellar Specific Expressed mRNA in Mouse:Subtract ive Hybridization by Phenol Emulsion Reassuciation Technique

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Abstract

There are extremdly complicated and numerous gene expressions in mammalian brain s-Each region of the brain contains a set of mRNA expressed only in that region and these mRNA would be related to presumed region of specific function.Genes pr eferentally expressed in cerebellum (CB) are therfore the reasonable candioate g enes for site of genetic lesions in CB neurodegenerative disorders.In this study ,the subtractive hybridization was introduced into screening and isolating CB sp ecific mRNA.Testing CB and front cortex (CTX) cDNA libraries were built,which co vered most low abundantly expressed mRNA.By applying over 100 fold excessive CTX and liver single strand cDNA to the CB double strand cDNA,subtractive hybridiza tion was carried out by phenol-emulsion-reassociation-technique (PERT),in which the comon expressed housekeep genes would be eluted by restrict site ligation,an d CB specific cDNA flanking with EcoRI site in both ds cDNA ends could be cloned into lamda gill phage.PERT subtraction reduced CB cDNA libraries from initial 5 .15×10 6 to remaining 2.5×10 2 recombinants,which were most likely CB specific expressed cDNA.Random Twenty recombinants (over 2 kb inserts) were amplified ra ndomly by polymerase chain reaction (PCR) and were hybridized with CB cDNA and l iver cDNA probes in dot blot.14 inserts positively hybridized with CB cDNA probe and only 3 inserts showed signals with liver cDNA probe.Colony blot presented s imilar results.10 cDNA clones which affinited to CB cDNA probe were selected for the Northern blot.In five tissures,2 cDNA clones binded only in CB mRNA channal ,6 were proved preferential expression in CB and low abundant expression in 4 other tissues,1 clone was a housekeep gene and 1 could not be detected in all tiss ues.The partially sequencing of clone PC7 and PD8 were introduced into Computer Gene Bank Data Base.No homologous complements were found between these two clone s and over ten thousands genes that have been sequenced.The two cloneswere newly reported.According to our experiment,we believe that:1)CB specific cally expres sed mRNAs are much less than expected comparing with other brain regions;2)most of CB mRNA expressions are in a preferential way other than unique expression.

Key words mRNA cDNA PCR Hybridization by PERT cDNA library Molecular hybridization

DNA seq uencing Mouse CB and CTX

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