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RNA 指纹法分离血管性痴呆大鼠海马内的差异表达基因

张雪朝 孙国杰

【摘要】目的 分离血管性痴呆(VD)大鼠海马内疾病相关基因。方法 用改良的 Pulsinelli 4-血管阻断全脑缺血法，4-VO 法建立 VD 模型，Morris 水迷宫检测其痴呆，以 RNA 指纹法对比正常老龄大鼠和血管性痴呆大鼠海马组织基因表达，并分离疾病情况下表达差异的基因。结果 在成功建立 VD 大鼠模型的基础上，分离、筛选、克隆到 32 条 VD 海马内疾病相关差异表达基因片段，选取 2 条 VD 拉特表达的片段进行 Northern 杂交验证，通过测序并与 GenBank 比较证实均为新基因片段，登录号为 BG937392, BG937393。结论 RNA 指纹法是快速、简便、有效的分离差异表达基因的方法，本实验分离的差异表达基因可能是直接参与疾病发生、发展过程的致病基因或保护基因。

【关键词】 痴呆, 血管性； 海马； 基因表达

Isolation of differently expressed cDNAs from the hippocampus of rats with vascular dementia by RNA fingerprinting ZHANG Xue-zhao, SUN Guo-jie, Department of Acupuncture, Hubei College of Traditional Chinese Medicine, Wuhan 430061

【Abstract】Objectives To isolate differently expressed cDNAs associated with vascular dementia from the hippocampus of rats. Methods The creation of a vascular dementia model of rat was by 4- vessel occlusion method. The learning and memory of vascular dementia(Vd) rats were examined by Morris water maze. RNA fingerprinting was used to analyse the differently expressed cDNAs in the hippocampus of normal aging and Vd rats. At the same time, cDNAs expressed differently during the progression of Vd were isolated. Results Thirty-two candidate cDNA fragments were isolated by RNA fingerprinting. The two cDNA fragments specifically expressed in Vd rats were analysed by Northern blot. Homology analysis through BLAST revealed that these two were novel genes and were given numbers of BG937392 and BG937393 in the gene bank. Conclusions RNA fingerprinting is a simple and quick method for isolating differently expressed genes. These two new gene fragments may contribute significantly to the process of Vd as pathogenic genes or protective genes.

【Key words】 Dementia, vascular; Hippocampus; Gene expression

血管性痴呆(VD)是指各种血管疾病引起的脑功能障碍而产生的获得性智能损害综合征，表现为记忆力、计算力、判断力、注意力、抽象思维能力、语言功能减退。【我们过去的实验【2】以及文献报道证实，VD 发病与一些基因表达的变化有关。基于 RNA 指纹法可筛选低丰度表达的差异基因，且所需起始材料少、假阳性率低，特别是它可以比较两组以上的 RNA 样本，故此我们于 2000 年 5 月至 2001 年 5 月用 4-血管阻断全脑缺血法建立老年大鼠 VD 模型，用 Morris 水迷宫检测其学习记忆障碍，而后用 RNA 指纹法筛选，克隆并鉴定海马组织中与 VD 大鼠模型相关的基因。

材料与方法

一、动物及取材

雄性 SD 大鼠，480 ～ 520 g，对照组、VD 组各 10 只。VD 组大鼠于 4-VO 术后 15 d 与对照组同期取材，断头处死，迅速取脑，置冰上分离海马，称重，置－70℃保存、备用。所用大鼠由上海中医药大学动物中心提供。

二、VD 模型的制备

采用改良的 4-VO 建立 VD 大鼠模型【3】。

三、行为学检测

术后第 10 d 按照相关文件【4】进行 Morris 水迷宫痴呆检测。

四、总 RNA 提取及鉴定

根据总 RNA 提取试剂盒(Ambion 提取试剂

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盒，US)说明书，采用异硫氰酸胍一步法提取海马组织中总 RNA。单链 cDNA 的合成根据试剂盒（delta differential display kit，CLONTECH）说明书操作。RNA 提纯法在 20 μl 反应体系中，加入 cDNA 1 μl，上述试剂盒提供的 T 及 P 引物各 1 μl，10× Klen Taq 酶缓冲液 2 μl，dNTP (5 mM) 0.2 μl，[α-32P]dATP (1 000 000 Ci/mM) 0.2 μl，Advantage Klen Taq 酶混合液 (50×) 0.4 μl，在 PTC-100 型 (MJ，US) PCR 扩增仪上，按下列条件进行反应：第一循环：94°C 变性 5 min，40°C 5 min，68°C 5 min，然后，94°C 变性 2 min，40°C 5 min，68°C 5 min 进行两个循环。94°C 变性 1 min，60°C 1 min，68°C 2 min 进行 25 个循环。反应结束后 68°C 延长 7 min。引物碱基序列：T1: 5'-CATATGCTGATATTCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
三、VD大鼠海马的mRNA的差别显示

以来自同一样本的两种不同浓度（1:10，1:40）的cDNA模板具有相同差异的条带为标准，在相同的背景条件下比较同一位置上的两组大鼠海马有差异的条带。初步筛选到32条差别条带，见图2。经过回收和再扩增，选取在VD组特异性表达的2条单一条带进行克隆。挑选单一白色菌落，扩增后提取质粒，酶切电泳，证实2个重组子的插入片段均与目的片段大小一致。经Northern Blot验证，见图2，差异片段得到证实，分别命名为VD2，VD5。

四、差别片段的序列分析

将2条有意义的差异片段用自动测序仪进行测序。

五、生物信息学分析将序列通过Internet与GENBANK DATABASE进行同源比较（Blast）

VD2，VD5未发现同源基因，为新的ESTs片段，此两条序列已在GENBANK DATABASE登录，登录号分别为：BG937392，BG937393。其序列分别为：（1）VD2片段大小为：398bp；（2）VD5片段大小为：210bp

讨 论

本实验在VD大鼠模型上成功地建立了RNA指纹法，分离出数十条血管性痴呆大鼠模型疾病相关基因，将VD特有的差异基因2条进行菌落筛选、克隆，进一步进行Northern Blot鉴定并得到证实。通过序列测定后与GenBankdatabase比较，未发现与本片段同源序列，提示可能是未知基因，分别命名为VD2，VD5，登录号为BG937392，BG937393。这两个疾病相关基因可能是直接参与疾病发生发展过程的致病基因或保护基因。VD2，VD5在体内其它部位的表达，基因cDNA全长序列以及其对蛋白质结构和功能的确认，我们正在做进一步研究。另外，实验中还在VD模型海马内发现了一些与VD疾病相关的上、下调基因片段，这些基因片段可能在VD发生、发展过程中有着十分重要的意义，我们现在正在对其进行分类、整理和鉴定。

目前对VD的研究多限于8d内短期观察，缺乏较长期的深入研究。海马是其研究涉及最多的结构，它是缺血损伤最敏感脑区，而且直接参与信息贮存和回忆。本实验在研究痴呆大鼠海马内基因的变化前首先进行了4-VO手术15d后大鼠学习记忆情况的观察。Volpe等（8）在1984年就通过实验证实，4-VO造成的全脑缺血所致的痴呆与人类临床脑组织缺血损伤所致痴呆相似，而且，它造成的学习记忆障碍是永久的，不可自行痊愈的。4-VO后的学习记忆成绩可为成功建立VD模型的客观指标（9）。本实验中4-VO手术15d后Morris水迷宫成绩显示，4-VO导致大鼠明显的空间记忆障碍，同时出现简单工作的障碍，这与近年其他实验研究结果一致（10）。

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