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Research Report

Dynamic variation of genes profiles and pathways in the hippocampus of ischemic mice: A genomic study

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ABSTRACT

Objective: To reveal the potential time-sequential molecular mechanism in the hippocampus of ischemia-reperfusion mice, so as to provide pertinent evidence for differential treatment during different phases after cerebral ischemia-reperfusion injury. *Methods* Seventy-five male Kunming mice were randomly divided into four groups: sham, ischemia and reperfusion for 3 h, 12 h, and 24 h, respectively. A cDNA microarray involving 374 cDNA ischemia-related genes, selected from the Science STKE database, was performed to detect the gene expression profiles. All data analyses were performed in the FDA ArrayTrack system. Data were also uploaded to the KEGG database (http://www.genome.jp/kegg/) to analyze the genetic pathways. *Results* Clustering and principal component analyses showed clear boundaries in the differentially expressed genes among the 3 h, 12 h, and 24 h groups. Although 56 overlapping up-regulated genes and 2 down-regulated genes were identified in 3 h, 12 h, and 24 h groups, the sequence variation of CA1 neurons and gene expression profiles also existed in all groups. Based on the total number of altered genes, the top 3 GO categories were metabolism, signal and cell cycle, which shared 8, 11 and 5 overlapping genes in 3 h, 12 h, and 24 h groups, respectively. As for metabolism, there were 2 specific altered genes in the 3 h group (casp8ap2 and mmp2), 6 in the 24 h group (daxx, gadd45a, adamts1, adcy8, cyp51, dusp16), but none in the 12 h group. Based on the KEGG database analysis, 18 overlapping pathways were detected in the three groups; and 1, 12 and 2 overlapping pathways were noted between the 3 h and 12 h, 12 h and 24 h, and 3 h and 24 h comparisons, respectively. The gene expressions of Caspase 2 and Rgs6 were identified by real-time RT-PCR, which was consistent with the results of microarray analysis. *Conclusion* Overlapping and variable genes and pathways demonstrate the time-sequential molecular mechanism in the hippocampus of ischemic mice, which may provide evidence for rational treatment during different phases after cerebral ischemia-reperfusion injury.

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1. Introduction

Cerebral ischemia-reperfusion injury would induce significant changes in the physiology, pathology and immune system within 24 h, and a large proportion of initially survived neuronal cells would die within the first few hours after reperfusion. Previous studies have shown that at 3 h after cerebral ischemia-reperfusion, infarction could be detected. And the infarction volume peaked at 24 h after ischemia-reperfusion injury, which was significantly larger than that at 3 h, 7 days, 14 days, and 21 days after injury. The infarction volume was significantly correlated with the score of neurological deficits (Min and Huai-lian, 2005). Therefore, it is demonstrated that 3 h and 12 h after ischemia-reperfusion injury are deemed to be the important time points, and early treatment within 24 h after injury is of great importance.

Secondary hemodynamic disturbances, the enhancement of inflammatory processes, free radical formation, vasogenic edema, and breakdown of blood-brain barrier (BBB) have been identified as factors that may contribute to the reperfusion injury by previous studies (T. Neumann-Haefelin et al., 2000; Wang, 1995). Studies also suggested that, these pathological changes by time sequence, e.g. early BBB damage consistently occurred after 2.5 h, appeared to be predictive of relatively pronounced transient increases in apparent T2 lesion size at subacute time points (1 to 2 days) (Neumann-Haefelin et al., 2000).

Since 1995 when gene expression spectrum was reported (Schena et al., 1995), gene chip technology has become an efficient and effective method to investigate gene expression profiles. Gene chip technology with high efficiency and reliability provides new methods for examining the changes of genes related to cerebral ischemia reperfusion injury (Lu et al., 2001; Zhou et al., 2003; Nagata et al., 2004). In recent years, enormous work, such as identification of possible genetic determinants of stroke risk and ischemic tolerance, as well as identification of differentially expressed mRNA (Keyvani et al., 2000), have already been done to understand the gene expression changes in the ischemic brain (Soriano et al., 2000). During ischemia-reperfusion, genes changed with time in accordance with the pathological changes. TIMP-1 mRNA has been identified to be significantly elevated at 24 h and 2 days after preconditioning, which corresponded well to the onset of ischemic tolerance (Wang et al., 1998). In healthy adult rats by MCAO, Caspase-3 protein was not activated at 1 h after ischemia-reperfusion, its expression level was elevated at 4 h, and continued to be elevated at 6 h and 12 h after injury (Ferrer et al., 2003). The expression levels of Bcl-2 and Bcl-xl were significantly elevated within the first 48 h and returned to baseline level at 7 days after injury (Wu et al., 2003).

The development of microarray technology allows the simultaneous measurement of the expression of many thousands of genes. The information gained offers an unprecedented opportunity to fully characterize biological processes. Pathway analysis, a promising tool to identify the mechanisms that underlie diseases, adaptive physiological compensatory responses and new avenues for investigation, involves looking for consistent but subtle changes in gene expression by incorporating either pathway or functional annotations. (Curtis et al., 2005). Researches on genetic pathways after ischemia-reperfusion injury have already been conducted. For example, at 1 h after ischemia-reperfusion injury, AMPK pathway was activated and reached its highest level (Li et al., 2009).

From what has been presented above, it could be concluded that cerebral ischemia is a dynamic pathological process involving multiple genes. And thus the change of a single gene expression cannot completely explain the pathological mechanism, and a genomic study on the time-dependent pathological mechanism of cerebral ischemia is needed. The relevant report, however, was found to be rare.

Studies have shown that the vulnerability of neurons to cerebral ischemia varies widely among different regions in the CNS. Different neuronal populations within a brain region also show different susceptibility to ischemic insult. Hippocampus is particularly vulnerable to ischemic insult (Pulsinelli et al., 1982). So based on the pathological process of cerebral ischemia and the special situation of hippocampus in cerebral ischemia, the gene expression profiles of ischemia-reperfusion and pathway analysis at 3 h (infarction detected), 12 h (between 3 h and 24 h) and 24 h (infarction volume peaked) in the hippocampus of ischemic mice were employed in our genomic study to reveal the dynamic pathological mechanism within 24 h after cerebral ischemia-reperfusion injury.

2. Results

2.1. *Time-dependent pathological variation on pyramidal cells in CA1*

Neuronal death at 3 h, 12 h and 24 h after reperfusion were assessed. Ischemia induced extensive death of pyramidal cells in CA1, and the few remaining pyramidal neurons were severely damaged at 3 h, 12 h and 24 h after reperfusion (Figs. 1b, c and d), resulting in cell loss that was quite different from that of normal tissue (Fig. 1a). These findings were validated by neuronal counts, which showed significant reduction in the number of neuronal counts from 3 h to 24 h after reperfusion (Fig. 1e).

2.2. *Altered gene expression profiles*

A total of 9, 5 and 20 differentially up-regulated genes were identified in the 3 h, 12 h and 24 h groups, respectively; and 1, 0 and 2 down-regulated ones were detected in the three groups, respectively. Fifty-six up-regulated and two down-regulated overlapping genes were identified among the three groups (Fig. 2 and Supplementary Table 1).

2.3. *Clustering analysis and PCA to differentially expressed genes*

The gene expression profiles of the 3 h, 12 h and 24 h groups displayed clear boundaries and emerged time sequence based on clustering analysis (Fig. 3). PCA showed that differentially expressed genes in the 3 h, 12 h and 24 h groups were significantly separated, which was consistent with the results of clustering analysis (Fig. 4).