Global Transcriptomic Profiling of Cortex and Striatum: Cerebral Injury after Ischemia/Reperfusion in a Mouse Model

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Objective: This study aims to investigate the molecular mechanism of injury development in the cortex and the striatum after cerebral ischemia/reperfusion (I/R). *Methods:* Gene expression data (GSE23160) in the cortex and the striatum of an intraluminal middle cerebral artery occlusion–I/R mouse model (N = 12) and sham controls (N = 4) were downloaded from the Gene Expression Omnibus. Limma package was used to identify the differentially expressed genes (DEGs) between the I/R (2, 8, and 24 hours) and control groups. Correlation analysis was then performed to identify the highly correlated differentially expressed genes (HCDEGs). STRING and Cytoscape software were used to construct a protein–protein interaction (PPI) network of HCDEGs. Furthermore, Venny 2.0 was used to identify common overlapped DEGs whose transcription factors (TFs) were predicted using iRegulon in Cytoscape. *Results:* For the cortex and the striatum, 2295 and 2282 DEGs were respectively identified between the I/R group and the controls, and were classified into 3 and 2 correlation modules. For each module, a PPI network was constructed, and Toll-like receptor 2 (Tlr2, degree = 25), interleukin 1β (Il1b, degree = 21), and heme oxygenase-1 (Hmox1, degree = 17) had high connective degrees. Furthermore, 29 common overlapped DEGs were found across time and tissue, which might be targeted by 13 TFs. Especially, Tlr2, Il1b, and Hmox1 were targeted by myeloblastosis protein (Myb, target count = 16) and FBJ osteosarcoma protein (Fos, target count = 15). Moreover, plasminogen activator urokinase receptor (Plaur) was targeted by Fos, and it was an HCDEG in correlation modules of both cortex and striatum. Upregulation of Tlr2, Il1b, Hmox1, and Plaur in I/R injury was confirmed using quantitative polymerase chain reaction and immunohistochemical staining. *Conclusion:* Tlr2, Il1b, Hmox1, and Plaur regulated by Myb and Fos might participate in cortex and striatum injury after cerebral I/R.
Key Words: Cerebral ischemia—differentially expressed genes—ischemia/reperfusion injury—transcription factor—weighted correlation network analysis.

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Introduction

Stroke is a major cause of death, adult chronic disability, and dementia.[1] Ischemic stroke accounts for approximately 80% of stroke cases, which might be caused by hypertension, dyslipidemia, lifestyle, and genetic factors.[1,2] After the onset of symptoms of acute ischemic stroke, recanalization therapies like intravenous tissue plasminogen activator and thrombectomy are generally performed to reperfuse the ischemic brain tissue and restore normal brain function. However, the ischemia/reperfusion (I/R) process in the brain can cause secondary damage, known as cerebral I/R injury. In addition to stroke treatment, cardiac arrest/resuscitation and neonatal hypoxic–ischemic encephalopathy can also cause I/R injury in the brain. Moreover, cerebral I/R injury is a major challenge during organ transplantation and cardiothoracic, vascular, and general surgeries. Understanding the development of injury after cerebral I/R may provide innovative therapeutic strategies for preventing injury after cerebral I/R or treating patients with cerebral I/R injury.

Various biological processes (BPs) are implicated in cerebral I/R, including vascular leakage, transcriptional reprogramming, no-reflow phenomenon, autoimmunity, innate and adaptive immune activation, and cell death.[3] During the process of cerebral I/R, the phosphorylated OxPhos components that control electron transfer, maintain $\Delta\Psi m$, maximize ATP production, and minimize reactive oxygen species generation are dephosphorylated, promoting reactive oxygen species generation and inducing mitochondrial dysfunction.[4] After cerebral I/R, cytochrome c that is released from mitochondria further induces neuronal cell death, in which necrotic and apoptotic events happen simultaneously.[5] Evidence has shown that caspases are activated in the brain following I/R.[5,6] Based on a transcriptomic microarray analysis, glutathione peroxidase-1, a crucial antioxidant enzyme, is found to play multifaceted roles in the development of injury after cerebral I/R via the p53-mediated proapoptotic pathway, Fas ligand-mediated pathways, Nrf2 (nuclear factor erythroid 2 p45-related factor 2) antioxidative cascade, and ubiquitin–proteasome system dysfunction.[7] However, that study mainly focuses on the roles of glutathione peroxidase-1. A more detailed analysis of the global transcriptomic profiling of the cortex and the striatum with and without cerebral I/R injury might indicate more information about the molecular mechanism underlying the development of injury in the cortex and the striatum after cerebral I/R.

In the present study, we comprehensively reanalyzed the gene transcriptomic microarray data (GSE23160[7]) in the cortex and the striatum with and without cerebral I/R injury (2, 8, and 24 hours after I/R) in a mouse model using bioinformatics methods. Briefly, differential expression analysis was performed to identify the genes with altered expression in the cortex and the striatum after cerebral I/R. Weighted correlation network analysis (WGCNA) was utilized to investigate correlations between these genes. Function enrichment analysis was conducted to investigate their functions. Protein–protein interactions (PPIs) were analyzed between these genes. Moreover, transcription factors (TFs) were predicted to find potential regulation pathways participating in the development of I/R injury. Expression changes of some key genes were confirmed by using quantitative reverse transcription–polymerase chain reaction (qRT-PCR) and immunohistochemical staining. Results of the present study might provide possible therapeutic targets to block the progression of injury in the cortex and striatum after cerebral I/R.

Methods

Gene Transcriptomic Profile

The microarray dataset of gene transcriptomic profiles, GSE23160,[7] was downloaded from the National Center for Biotechnology Information-Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23160). GSE23160 contained 16 right cortex and 16 right striatum samples of the *Mus musculus*. For the I/R group, suture-induced middle cerebral artery occlusion (MCAO) was kept for 2 hours, and reperfusion was then performed. Cortex and striatum tissues corresponding to the infarct region were obtained 2 hours (n = 4; cortex-2h, striatum-2h), 8 hours (n = 4; cortex-8h, striatum-8h), and 24 hours (n = 4; cortex-24h, striatum-24h) post I/R, as well as sham controls (n = 4; cortex-sham, striatum-sham). Microarray data were generated based on the platform of GPL6885 (Illumina MouseRef-8 v2.0 Expression BeadChip; Illumina Inc., San Diego, CA, USA).

Data Preprocessing

The non-normalized data were preprocessed using limma (version 3.26.3, R package)[8] provided by online service Bioconductor (http://www.bioconductor.org/). A transcription matrix was obtained after background correction, data normalization, expression calculation, probe summarization, and probe identifier conversion into gene symbols.

Differential Expression Analysis

To obtain the differentially expressed genes (DEGs) between the I/R and control groups, differential expression analysis was performed for cortex and striatum samples using the limma package.[8] A total of 6 DEG sets were generated, including cortex-2h versus cortex-sham, cortex-8h versus cortex-sham, and cortex-24h versus cortex-sham; and striatum-2h versus striatum-sham, striatum-8h versus striatum-sham, and striatum-24h versus striatum-sham. During this analysis, nonpaired *t*-test in