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Altered expression of long non-coding RNA and mRNA in mouse cortex after traumatic brain injury

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ABSTRACT

Background and objective: The present study aims to detect the altered lncRNA expression in the mouse cortex after traumatic brain injury (TBI). We also simultaneously detected the altered mRNA profile to further analyze the possible function of lncRNA.

Method: C57BL/6 mice (n=18) were used to construct a controlled cortical impact model. At 24 h post-TBI, the cortex around injury site was collected and the total RNA was extracted to construct the cDNA library. RNA sequencing (RNA-seq) was carried out followed by RT-PCR for confirmation. Bioinformatic analysis (including GO analysis, KEGG pathway and co-expression analysis) also were performed.

Results: A total of 64,530 transcripts were detected in the current sequencing study, in which 27,457 transcripts were identified as mRNA and 37,073 transcripts as lncRNA. A total of 1580 mRNAs (1430 up-regulated and 150 down-regulated) and 823 lncRNAs (667 up-regulated and 156 down-regulated) were significantly changed according to the criteria ($|\log_2^{(\text{fold change})}| > 1$ and $P < 0.05$). These altered mRNAs were mainly related to inflammatory and immunological activity, metabolism, neuronal and vascular network. The expression of single lncRNA may be related with several mRNAs, and so was the mRNA. Also, a total of 360 new mRNAs and 8041 new lncRNAs were identified. The good reproducibility and reliability of RNA-seq were confirmed by RT-PCR.

Conclusion: Numerous lncRNAs and mRNAs were significantly altered in mouse cortex around the injury site 24 h after TBI. Our present data may provide a promising approach for further study about TBI.

1. Introduction

Due to the limited number of effective therapeutic strategies available for traumatic brain injury (TBI), TBI still represents the leading cause of morbidity and mortality in individuals under the age of 45 years (Werner and Engelhard, 2007), resulting in severe economic burden for families and societies (Loane and Faden, 2010). The pathophysiological mechanisms of TBI are complex and associated with altered gene expression. Several studies (Long et al., 2003; Matzilevich et al., 2002; Raghavendra Rao et al., 2003; Kobori et al., 2002) suggest that TBI greatly alters the expression of protein-coding genes, mainly involving inflammatory reaction, oxidative stress, apoptosis, metabolism, cellular repair and plasticity.

It is clear that ~90% of eukaryotic genomes are transcribed (Wilhelm et al., 2008), and only 1–2% of the genome encodes proteins (Birney et al., 2007), suggesting that the majority of transcripts represent non-coding RNA (ncRNA). The ncRNA consists of two major classes: the small ncRNA, which is less than 200 nucleotides including microRNA (miRNA), and long noncoding RNA (lncRNA), which is a novel class of non-protein coding transcripts longer than 200 nucleotides (Jiang et al., 2015; Mercer et al., 2009).

LncRNAs were initially considered as transcriptional by-products. However, the accumulated evidence suggests a key role of lncRNA in various biological processes. Recent data suggests that lncRNA regulates the gene expression by interfering with transcription, post-transcriptional processing, and chromatin remodeling (Bali and Kuner, 2014; Batista and Chang, 2013). LncRNAs are highly expressed in the CNS. Their expression profiles are associated with specific neuroanatomical regions, cell types, or subcellular compartments suggesting a potential functional role in the nervous system (Qureshi and Mehler, 2012; Ramos et al., 2013; Mercer et al., 2008). The aberrant expression of lncRNA has been

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shown to be associated with a variety of central nervous system diseases, such as Alzheimer's (Faghihi et al., 2008), and Huntington's diseases (Johnson, 2012). LncRNA also has been reported to mediate synaptogenesis (Bernard et al., 2010), axon injury (Yu et al., 2013), neurogenesis (Ng et al., 2013), and neuronal differentiation (Ng et al., 2012). Axon injury, synapse remolding and neural regeneration are important processes in TBI pathophysiology. The current data suggest that lncRNA may mediate TBI and play an important role in its pathological process.

Altered miRNA expression in cortex (Lei et al., 2009), hippocampus (Liu et al., 2014) and serum (Sharma et al., 2014) after TBI has been reported. However, the global lncRNA expression profile in TBI is poorly defined. Therefore, in the present study, we explored the lncRNA expression profile in the cortex of normal and TBI-injured mice 24 h post-TBI by RNA-sequencing (RNA-seq). In order to explore the function of altered lncRNA, we also measured the mRNA expression profile. The enriched pathway, biological process, cellular components and molecular function were explored using bioinformatics. The relationship between the differentially expressed lncRNAs and mRNAs was also analyzed by constructing the co-expression network.

2. Results

2.1. Mapping of sequencing reads

All the reads were optimized and the clean data were mapped to mouse chromosomes. All the samples from both normal and TBI groups exhibited very similar chromosome coverage and DNA distribution. In view of the similarity between several sequencing data, we presented the representative data from both normal and TBI groups, and the detailed information were available as supplemental data (supplement 1). In the sample representing the normal group, the total detected reads were 100,290,212 (100%), total mapped reads were 96,850,597 (96.57%), multiple mapped reads were 6,462,949 (6.44%), uniquely mapped reads were 90,387,648 (90.13%), reads mapped in proper pairs were 47,712,682 (47.57%), reads mapped to "+" strand were 48,643,763 (48.50%), and reads mapped to "-" strand were 48,206,834 (48.07%). All the reads were aligned to mouse chromosomes (chr1–19, X and Y) (Fig. 1, A) and centered on several different regions: exons (74.99%), introns (19.47%), intergenic region (5.28%), and splicing region (0.26%) (Fig. 1, B). In the sample representing the TBI group, all the sequencing reads were aligned to mouse chromosome (chr1–19, X and Y) (Fig. 1, C), and the total

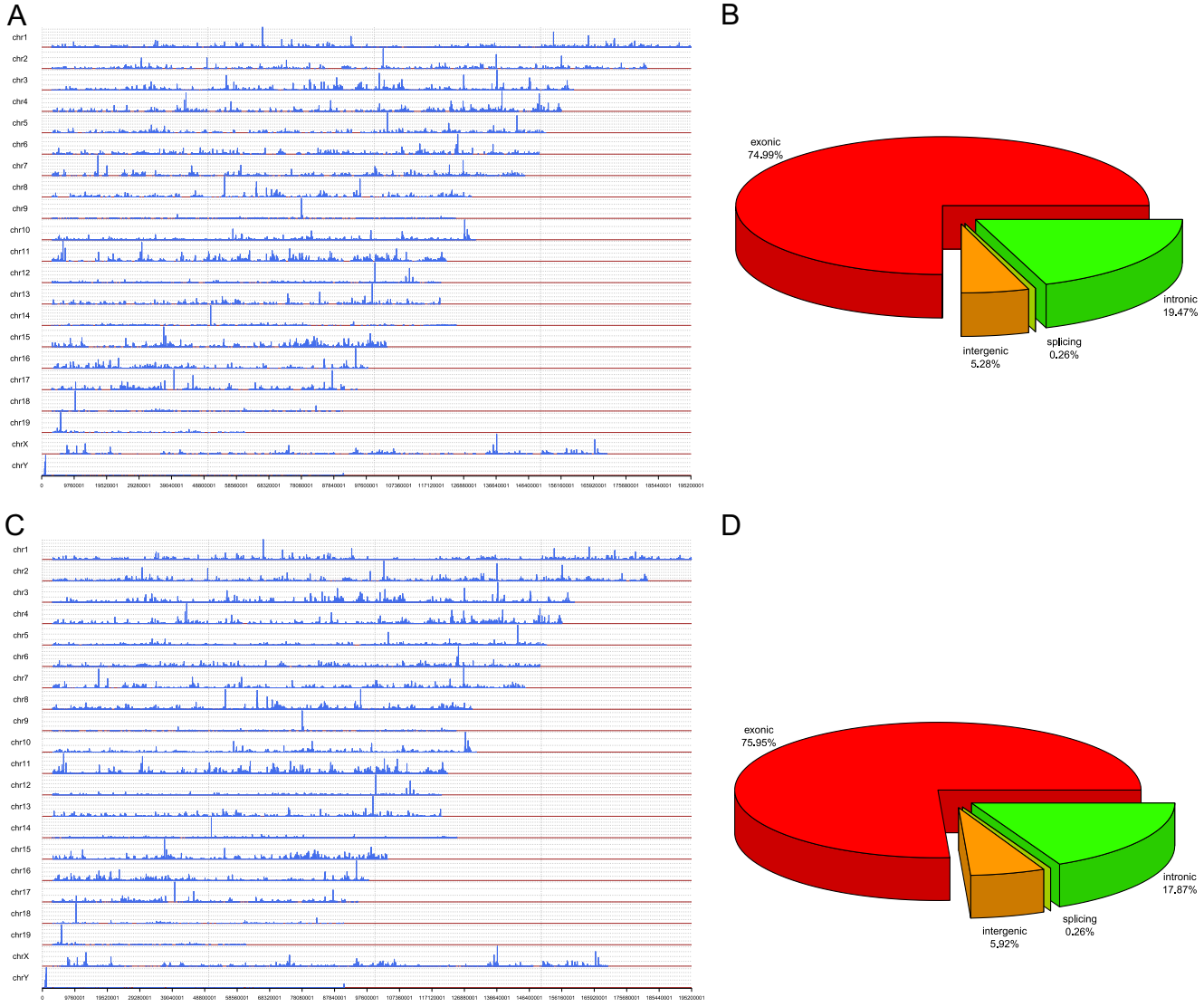

Fig. 1. Representative mapping of sequence reads in the normal and TBI groups. "A" and "C" represented the overall coverage of the detected reads on mouse chromosome, and "B" as well as "D" represented the general distribution of reads in different DNA regions. "Chr" in "A" and "C" refers to chromosome.