available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Differential expression and alternative splicing of genes in lumbar spinal cord of an amyotrophic lateral sclerosis mouse model

Huifang Chen[a], *Yansu Guo*[a,b], *Ming Hu*[a], *Weisong Duan*[a,b], *Geng Chang*[a], *Chunyan Li*[a,b,*]

[a]*Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 05000, People's Republic of China*
[b]*Hebei Province Key Laboratory of Neurology, Institute of Cardiocerebrovascular Disease, Shijiazhuang, Hebei 05000, People's Republic of China*

ARTICLE INFO

Article history:
Accepted 21 March 2010
Available online 31 March 2010

Keywords:
Amyotrophic lateral sclerosis
Exon array
Inflammation
Cell adhesion
Immune

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is one of the most common adult-onset neurodegenerative diseases, with progressive paralysis and muscle atrophy. The exact pathogenic mechanism remains unknown, but recent evidence suggests that differential gene expression and gene splicing may play a significant role. We used Affymetrix GeneChip® Mouse Exon 1.0 ST Array to investigate the expression profiling of lumbar spinal cord samples from SOD1-G93A transgenic mice, the widely used animal model of ALS. The de-regulated genes analyzed either from the expression level or from the alternative splicing level both showed overlapping GO categories and pathway mapping. Our findings indicate that cell adhesion, immune-inflammation response and lipid metabolism all play important roles in the onset of ALS. Detailed analysis by RT-PCR of key genes confirmed the experimental results of microarrays. These results suggest a multi-factor mechanism in ALS development.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is one of the most common adult-onset neurodegenerative diseases, characterized by progressive and relatively selective degeneration of the upper and lower motor neurons in the spinal cord, brainstem, and motor cortex (Shaw, 2005; Ince et al., 1998). Patients have progressive paralysis and muscle atrophy, and half of the patients die on average within 3 to 5 years of symptom onset, mostly resulting from respiratory failure. The global incidence of ALS is about 1–2 per 100,000 each year and the lifetime risk of developing ALS is estimated to be approximately 1/600 to 1/2000. (Shaw, 2005; Pasinelli and Brown, 2006).

Ninety percent of ALS are sporadic and the remaining 10% is familial, but the two forms of ALS are clinically and pathologically indistinguishable (Andersen, 2006). Of the familial cases, 20% are associated with a mutation in the cytosolic copper/zinc superoxide dismutase 1 gene (hSOD1) (Rosen et al., 1993). The

* *Corresponding author*. Department of Neurology, The Second Hospital of Hebei Medical University, Institute of Cardiocerebrovascular Disease, 215 Hepingxi Road, Shijiazhuang, Hebei 050000, People's Republic of China. Fax: +86 311 87064024.
E-mail address: chunyanli5@yahoo.com.cn (C. Li).

Abbreviations: ALS, amyotrophic lateral sclerosis; SOD1, copper/zinc superoxide dismutase 1; HC, hierarchical cluster; GO, gene ontology; MAS, molecule annotation system; KEGG, Kyoto Encyclopedia of Genes and Genomes; SI, splicing index; NI, gene-level normalized intensity; LPL, lipoprotein lipase; PDCD1, programmed cell death 1; ECM, extracellular matrix

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.03.075

SOD1 mutations are predominantly missense mutations leading to substitution of one amino acid by another, for example glycine by alanine (hSOD1-G93A). Because expression of a hSOD1 mutant in animals causes an adult-onset phenotype recapitulating closely the human disease both clinically and histopathologically, these transgenic animals have become a widely used ALS model. In fact, the effects of the mutant are due to the toxic gain of function, while most of SOD1 mutants retain enzymatic activity. Yet, at the present time, the actual mechanism by which mutant SOD1 initiates ALS is poorly understood, although several hypotheses have been proposed to explain the toxic effect of mutated SOD1, on the basis of extensive research on ALS: oxidative stress, glutamate excitotoxicity, formation of high-molecular-weight aggregates, mitochondrial dysfunction and apoptosis (Beckman et al., 2001; Cleveland and Rothstein, 2001; Bruijn et al., 2004; Manfredi and Xu, 2005; Kirkinezos et al., 2005).

The availability of powerful genomic technologies provides the opportunity to unravel complex regulatory and disease mechanisms. Alternative splicing of mRNA transcripts is a major form of post-transcriptional gene regulation, during which introns in a pre-mRNA are differentially removed and exons are joined to form multiple forms of mature RNA. Alternative splicing plays an important role in generating proteomic diversity, as a relatively limited number of genes may expand into very complex proteomes. It has been estimated that about three-quarters of all human genes exhibit alternative splicing (Johnson et al., 2003; Wang et al., 2008). Current data suggest that greater than 90% of all human genes undergo alternative splicing (Pan et al., 2008; Wang et al., 2008). Statistic report of ASTD database *Mus musculus* (http://www.ebi.ac.uk) shows that 57% genes undergo alternative splicing. Researchers are becoming aware of the dominant role of mRNA splicing in many important physiological processes such as development, physiology and disease (Johnson et al., 2003). Affymetrix Exon Arrays are rapidly gaining popularity and becoming a standard for both gene- and exon-level expression analysis (Gardina et al., 2006; Yeo et al., 2007; Thorsen et al., 2008; Hang et al., 2009; Xi et al., 2008; McKee et al., 2007).

Recent studies have shown that alternative splicing is especially prevalent in the nervous system. Zhang et al. (2009) reported that aberrant cleavage of TDP-43 enhances aggregation and cellular toxicity in ALS. The glutamate transporter 1 in rodents, or EAAT2 in humans, is alternatively spliced in a complex manner, including the use of multiple 5′ and 3′ untranslated regions and several coding variants (Lauriat et al., 2007). Though alternative splicing is prevalent in the nervous system, little is known in ALS.

In the present study, we used Affymetrix GeneChip® Mouse Exon 1.0 ST Array to identify genes and exons that are differentially expressed in SOD1-G93A transgenic mice. The array contains approximately 5.4 million probes grouped into 1.4 million probe sets and interrogates more than 1 million known and predicted exons. For each gene, the median number of probes is 30 to 40, usually distributed along the entire transcript sequence. The commercial availability of specific software provides a powerful tool for the study of alternative splicing (exon-level expression analysis) and interrogation of the expression of every known and predicted exon. At the same time, exon arrays provide robust gene-level expression analysis (Affymetrix Inc., 2006).

For the first time, we have identified not only genes that are altered in expression in response to mutant SOD1, but also genes that undergo alternative splicing. The study was focused on the lumbar spinal cord which is the most affected tissue in the SOD1-G93A transgenic mice.

2. Results

2.1. *Assessing disease onset in the SOD1 mouse model of ALS*

Our experiments show transgenic mice C57BL/6J-SOD1-G93A develop a neurodegenerative disease resembling human ALS. These mice at birth appear identical to the non-transgenic littermate control group. The transgenic animals were considered to be at onset stage when they had behavioral symptoms, lost 10% of their body weight, or their performance began to decline steadily in the rotarod test, whichever occurred first.

2.2. *Gene-level analysis*

2.2.1. *Differentially expressed genes*

Using the mouse exon 1.0 ST, we analyzed the transcription profiles of the lumbar spinal cord removed from three female SOD1-G93A mice at the onset of the disease and three female non-transgenic G93A littermates. Based on the three biological replicates, 14,318 transcripts on the chip passed our signal threshold for subsequent analyses. To identify genes differentially expressed in the presence of SOD1-G93A, comparisons were made between the transcription profiles of lumbar spinal cord from the transgenic mice and non-transgenic littermates. We selected transcripts that showed an increase/decrease of at least two-fold with a statistical significance of $p<0.05$, and a difference in signal intensity between the baseline and experimental arrays of at least 50 U of fluorescence.

The analyses identified a total of 322 transcripts that were statistically differential in the presence of the mutant SOD1 protein: 309 (95.96%) transcripts were up-regulated and 13 (4.04%) transcripts were down-regulated. Thus, 322 of the 14,318 (2.2%) transcripts detected by the array were differentially expressed in the presence of the mutant SOD1. The number of genes showing an increased expression is far greater than those showing a decrease in expression. The number of up-regulated genes is 23.77 (309/13) times higher than the number of down-regulated genes. It appears that expression of mutant SOD1 within lumbar spinal cord leads to a marked degree of transcriptional increase at disease onset. Based on *T*-test of the results, we generated a valcano plot to compare visually the differentially expressed genes between the onset group and the control group (Fig. 1). Tables 1 and 2 list top 90 up-regulated and bottom 10 down-regulated genes.

2.2.2. *Cluster analysis of differentially expressed genes*

The source of variation which is present in the exon array study can be visualized by hierarchical cluster (HC) analysis. Cluster analysis 3.0 software was used for HC analysis. HC