Amyotrophic lateral sclerosis (ALS) progression: searching for predictive microRNAs in SOD1G93A mice

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Abstract
Evaluation of muscle and plasma miRNA expression in SOD1G93A mice in order to identify potential biomarkers for ALS, aiming at new targets for therapeutic approaches.

Key words: ALS, miRNA, biomarker

Introduction

Amyotrophic lateral sclerosis (ALS) is a lethal motoneuron disease that progressively debilitates sick individuals. The diagnostic process relies on a series of examinations to rule out alternative disorders with a similar presentation. There is no cure for ALS and the only approved disease-modifying drug, Riluzole, only modestly slows the evolution to death. Therefore, biomarkers are greatly needed to facilitate ALS diagnosis and prognosis, indicating the potential success of new therapy trials. As a potential candidate as biomarkers, microRNAs (miRNAs), small posttranscriptional modifiers of gene expression, are frequently altered in disease conditions. Besides their important regulatory role in a variety of biological processes, miRNAs can also be upregulated in the plasma due to pathological disorders. In order to find biomarkers for ALS, we have studied miRNA alterations (miR-206, miR-9, miR-424, miR-886-5p) at skeletal muscle and plasma of male and female SOD1-G93A mice.

Results and Discussion

The samples were obtained from SOD1-G93A strain, from presymptomatic (70days), symptomatic (100days) and terminal stage mice (=140days). Blood was extracted through the left ventricle, and plasma was collected. Immediately after the blood extraction, the muscles were dissected out (right and left tibialis anterior) and the samples were stored at -80°C. The total RNA from plasma was extracted using miRVana (Ambion, Life Technologies) according to kit instructions. Trizol was used for muscle RNA extraction. The quantity and quality of each extraction was obtained through NanoPhotometer (Implen), and the integrity was assessed through agarose gel 1%. The cDNA was synthesized using Taqman miRNA Reverse Transcription kit, with specific primers, according to the manufacturer instructions. According to 260/280 and 260/230 ratios, the samples were not contaminated with any reagents, carbohydrates or proteins (Chart 1).

The selected candidate miRNAs will be investigated individually with quantitative PCR in the tibialis anterior muscle and plasma.

To date, the RNA extraction and cDNA synthesis was done, for later qRT-PCR.

Image 1. Agarose Gel (1%) to assess RNA Integrity.

Although it was observed contamination with genomic DNA in some samples, it wasn’t necessary the treatment with DNase, because it was used specific primers with the kit Taqman miRNA Reverse Transcription for cDNA synthesis.

Chart 1. Representation of concentration, purity and quality of samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>ng/µL</th>
<th>260/280</th>
<th>260/230</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>294</td>
<td>1.83</td>
<td>2.27</td>
</tr>
<tr>
<td>2</td>
<td>288</td>
<td>1.83</td>
<td>2.20</td>
</tr>
<tr>
<td>3</td>
<td>210</td>
<td>1.80</td>
<td>2.25</td>
</tr>
<tr>
<td>7</td>
<td>265</td>
<td>1.79</td>
<td>2.19</td>
</tr>
</tbody>
</table>

Samples were considered valid when values of concentration were above 100ng/µL and ratios between 1.7 and 2.5.

Conclusions

We expect that the quantification of the mentioned miRNAs will be used as a tool for diagnosis and prognosis in patients with ALS.

Acknowledgement

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