MicroRNA identity and abundance in porcine skeletal muscles determined by deep sequencing

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INTRODUCTION

MicroRNAs (miRNA) are short single-stranded RNA molecules that regulate gene expression posttranscriptionally by binding to complementary sequences in the 3' untranslated region of target mRNAs. Identification of the complete set of miRNAs expressed in muscles is likely to significantly increase our understanding of muscle growth and development.

RESULTS and DISCUSSION

To determine the identity and abundance of miRNA in porcine skeletal muscle we applied a deep sequencing approach using Illumina Genome Analyzer Sequencing. This allowed us to identify the sequences and relative expression levels of 212 miRNA genes, thereby providing a thorough view of the complete set of miRNAs expressed in muscles is likely to significantly increase our understanding of muscle growth and development.

The sequencing data for two prominent genes, miR-133 and miR-206, were validated by Northern blotting (Fig. 2). The results confirm that both are highly expressed in muscle tissues but undetectable in brain samples. Furthermore, miR-133 revealed two bands in agreement with the sequencing data, showing that miR-133 is heterogeneous in length with 22 and 23 nucleotides being the most abundant mature transcripts. Quantitative RT-PCR (not shown) confirmed that miR-206 was expressed at higher levels than miR-133 in muscle tissues and that miR-133 also has low-level expression in non-muscle tissues.

The expression profiles of miR-133 and miR-206 were examined during muscle cell growth in culture (Fig.3). Both miRNAs were strongly upregulated during differentiation of myoblasts along the myogenic pathway into myotubes. Consistently, miR-133 and miR-206 are known to be highly expressed during differentiation of myoblasts along the myogenic pathway into myotubes. Consistently, miR-133 and miR-206 are known to be highly expressed in muscle tissues but undetectable in brain samples.

Table 1: Identity of the top 50 most abundant porcine miRNAs in skeletal muscle tissue (longissimus dorsi)

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Count</th>
<th>miRNA</th>
<th>Count</th>
<th>miRNA</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-1</td>
<td>29664567</td>
<td>miR-7a</td>
<td>1893155</td>
<td>miR-133</td>
<td>54477</td>
</tr>
<tr>
<td>miR-133</td>
<td>107235</td>
<td>miR-13a</td>
<td>83335</td>
<td>miR-206</td>
<td>73255</td>
</tr>
<tr>
<td>miR-423</td>
<td>4533</td>
<td>miR-13b</td>
<td>5345</td>
<td>miR-203</td>
<td>63335</td>
</tr>
<tr>
<td>miR-203</td>
<td>33455</td>
<td>miR-22-3p</td>
<td>9486</td>
<td>miR-30a-5p</td>
<td>1086</td>
</tr>
<tr>
<td>miR-30a-5p</td>
<td>5894</td>
<td>miR-204-3p</td>
<td>24755</td>
<td>miR-30b-5p</td>
<td>1086</td>
</tr>
</tbody>
</table>

PicTar and TargetScan were used to predict target mRNAs of the ten most abundant miRNAs (Table 2). The predicted targets (1984 genes) were classified according to KEGG functional annotations to identify pathways that are regulated by miRNAs (Table 2). The MAPK signalling pathway is involved in regulation of muscle differentiation by affecting the activities of myogenic transcription factors as well as by controlling the expression of structural muscle genes. The Wnt signalling pathway promotes adult skeletal muscle regeneration from satellite cells and induces myogenesis in the somites. Pathways associated with cancer suggest a role in cell cycle progression and cell proliferation. Axon-guidance and long-term potentiation pathways suggest participation in the function of the nervous system. Pathways associated with focal adhesions, ECM-receptor interaction and regulation of the actin cytoskeleton indicate a role in cell motility, communication between cells and the extracellular matrix. These results illustrate some of the possible roles of the highly expressed miRNAs in muscle biology.

Table 2: KEGG pathways enriched for targets of the ten most abundant miRNAs expressed in porcine muscle tissue

<table>
<thead>
<tr>
<th>Pathway</th>
<th>miR-133</th>
<th>miR-206</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axon guidance</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>MAPK signalling pathway</td>
<td>62</td>
<td>34</td>
</tr>
<tr>
<td>Focal adhesion</td>
<td>49</td>
<td>25</td>
</tr>
<tr>
<td>ECM-receptor interaction</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>Wnt signalling pathway</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Long-term potentiation</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Regulation of actin cytoskeleton</td>
<td>46</td>
<td>46</td>
</tr>
</tbody>
</table>

Figure 1. Hairpin structures, sequence and length heterogeneity of ssc-miR-423.

Figure 2. (A) Northern blot of ssc-miR-133 and ssc-miR-206. RNA smaller than 200 nt were electrophoresed on 15% polyacrylamide gel under denaturing conditions, blotted and hybridized with 32P-end labelled DNA oligonucleotide probes. Lane 1, frontal cortex; lane 2, cerebellum; lane 3, triceps; lane 4, longissimus dorsi. (B) Size histogram of sequenced ssc-miR-133 and ssc-miR-206 respectively.

Figure 3. Expression profiles of miR-133 and miR-206 in differentiating myoblast cells. The expression of the miRNAs are normalized to the reference gene GAPDH. The analysis is performed on cultured myoblasts grown in growth media from day 0 to day 2, and in differentiation media from day 3 to day 5.