

# *MicroRNA: Small RNAs that have a big impact on skeletal muscle development and growth*

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## INTRODUCTION

MicroRNA (miRNA) are noncoding small RNAs, 18-26 nucleotides long, that regulate gene expression by altering translation of protein encoding transcripts (Hutvagner et al., 2005). As a result of decreased translation, miRNA guide developmental decisions including cell fate, cell cycle progression, apoptosis, adipocyte differentiation, and processes that alter muscle development and growth (Brennecke et al., 2005; Carthew 2006; Kajimoto et al., 2006; Callis et al., 2007). Functionally important small RNAs were first described in nematodes in 1993. However, it was not until 2001 that researchers began to understand the function of this family of RNAs that includes miRNA, and to recognize their significance was not confined to lower order organisms. With the increase interest in the role of miRNA in cellular processes that impact animal biology, research has expanded to determine what role miRNA have in various livestock species. Therefore, this presentation will focus on the current literature evaluating the role of miRNA in skeletal muscle development and the on-going research in our lab profiling miRNA abundance in skeletal muscle of developing swine and identifying predicted genes that these miRNA target.

## ROLE IN SKELETAL MUSCLE

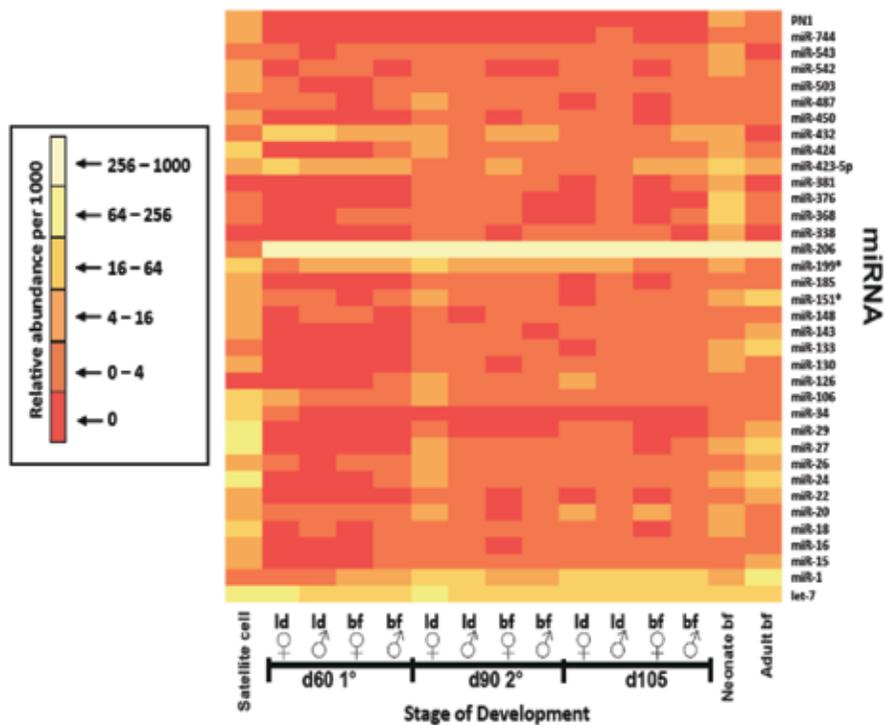
MicroRNA were initially reported to have a role in skeletal muscle development utilizing mouse, drosophila, and zebrafish models. Three muscle-specific miRNA (miR-1, miR-133, and miR-206) that undergo an increase in abundance during muscle cell differentiation were initially identified (Brennecke et al., 2005; Chen et al., 2006). However, these miRNA have been reported to regulate different stages of myogenesis. MiR-133 increases proliferation of C<sub>2</sub>C<sub>12</sub> myoblasts, whereas miR-206 and miR-1 promote differentiation (Chen et al., 2006). Research

in livestock models has also begun to evaluate the role these miRNA have in skeletal muscle development. For example, muscle-specific miRNA have been reported to regulate a gene that directly impacts skeletal muscle development and growth in sheep (Clop et al., 2006). Specifically, a mutation in the myostatin gene of heavily muscled Belgian Texel sheep creates a target site for miR-1 and miR-206 in the exon encoding the 3' UTR of the transcript, resulting in decreased translation of the myostatin protein and consequent increase in muscle mass.

## ABUNDANCE OF MICRORNA IN SWINE SKELETAL MUSCLE DURING DEVELOPMENT

To evaluate the role of miRNA in skeletal muscle of swine, we initially evaluated global miRNA abundance at specific developmental stages in swine (McDaneld et al., 2009). Time points evaluated included proliferating satellite cells (4th, 5th, and 6th passage), three stages of fetal development (60, 90, and 105 day-old fetus), day-old neonate, and adult. A digital transcriptome profile approach was applied to evaluate miRNA abundance based on cloning the miRNA population from each sample and evaluating abundance as the number of transcripts for a given miRNA gene per thousand transcripts observed. Upon evaluation of the miRNA abundance profiles, twelve potential novel miRNA for swine were detected that did not match previously reported sequences in the miRNA database. In addition, a number of miRNA previously reported to be expressed in mammalian muscle were detected, having a variety of abundance patterns through muscle development (Figure 1). Muscle-specific miR-206 was nearly absent in proliferating satellite cells in culture, but was the highest abundant miRNA at other time points evaluated. In addition, miR-1 was moderately abundant throughout developmental stages with highest abundance in the adult. In contrast, miR-133 was moderately abundant in adult muscle and either not detectable or lowly abundant throughout fetal and neonate development. In addition to muscle-specific miRNA, a larger number of ubiquitous miRNA were present across all libraries.

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**Figure 1. Abundance of miRNA in swine skeletal muscle at various developmental time points. Longissimus dorsi = Lf, Biceps femoris = bf.**

## IDENTIFICATION OF MICRORNA GENE TARGETS

MicroRNA regulate gene expression by inhibiting gene translation through targeting of a miRNA-protein complex by base-pairing of the miRNA sequence to cognate recognition sequences in the 3' UTR (untranslated region) of the mRNA. Target identification for a given miRNA sequence is generally accomplished by informatics analysis of predicted mRNA sequences present in the genome or in databases of transcript sequence for the tissue of interest. However, gene models for porcine skeletal muscle transcripts in current databases are inadequate for this exercise.

To provide data necessary to identify gene targets for miRNA that we previously identified to be expressed in porcine skeletal muscle, cDNA libraries from swine skeletal muscle were sequenced using Roche 454 GS-FLX next-generation pyrosequencing and a *de novo* assembly of transcripts enriched in the 3' UTR was performed using a sequence assembly program. Over 725 million bases of sequence were generated, which assembled into 18,202 sequence contigs that were compared to mRNA and protein databases. Sequence reads were also mapped to a 3' UTR database containing porcine sequences. The identified 3' UTR were then examined to predict targets for previously identified miRNA that had been separately sequenced from the same porcine muscle sample used to generate the cDNA libraries.

A number of the miRNA-targeted genes were associated with signaling pathways that impact skeletal muscle growth and function, including calcium ion binding, actin structure, and insulin signaling pathway (Clemmons, 2009; Perrini et al., 2010). Additionally, several signaling pathways previously reported to have a crucial role in mediation of skeletal muscle development were associated with miRNA-targeted genes including JAK-STAT (janus kinase-signal transducer and activator of transcription), MAPK (mitogen activated protein kinase), mTOR (mammalian target of rapamycin), PPAR (peroxisome proliferator-activated receptor), TGF- $\beta$  (transforming growth factor-beta), and Wnt signaling pathways (Keren et al., 2006; Yoon et al., 2008; Miyazaki and Esser, 2009; Trenerry et al., 2011).

## CONCLUSIONS

MicroRNA are important regulators of gene expression that impact biological pathways. The data presented herein identify miRNA that may have a role in progression of myogenesis throughout development and their function may be specific to different stages of skeletal muscle growth. Additionally, through *de novo* reconstruction of transcripts expressed in skeletal muscle, our results identify genes targeted by highly expressed miRNA in porcine skeletal muscle. While we identified a number of miRNA that are highly expressed in skeletal muscle and target 3' UTR of transcripts expressed in skeletal muscle, there is a large portion of these expressed miRNA that have not been

previously reported to regulate skeletal muscle growth. This suggests that further research needs to be completed to expand our understanding of how these miRNA target genes that regulate skeletal muscle growth.

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