Dear Editor,

Thank you for informing us that our study has the potential to be published by “**PeeJ**”. We sincerely appreciate the thoughtful and constructive comments from the reviewer, and your assistance in improving the manuscript. We have gone through all the reviewers’ comments in detail and believe that we have fully addressed their questions and concerns. Below we provide a copy of the reviewer’s comments with our point-to-point responses.

We look forward to hearing a positive response from you.

Best regards,

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**Reviewer 1**

**Basic reporting**

The manuscript describes a comprehensive investigation of miRNA transcriptomes by RNA sequencing across five porcine muscle development stages including one prenatal stage and four postnatal stages which represented prenatal, natal, weaning, young adult, and middle-aged stages, respectively. The authors identified 404 known porcine miRNAs, 118 novel miRNAs, and 101 miRNAs that are conserved in other mammals and a set of universally abundant miRNAs common to all the development stages. They suggest that this miRNAs may play housekeeping roles in myogenesis. In addition, they describe enhanced expression levels of miRNAs related to differentiation and morphogenesis in the embryonic stage and miRNAs related to apoptosis later in muscle development. Some differentially expressed miRNA were validated by an independent method (qPCR). The manuscript is written in good English, the background and results/discussion section well balanced and the figures sufficiently described.

**Experimental design**

The experimental design is explained clearly, data well described and rigorously conducted and methods sufficiently detailed for reproducibility.

**Validity of the findings**

The findings appear robust and statistically sound. The data described highlight the importance of miRNA during pig muscle development and represent a source of data for future research.

Response:

Thank you for these positive remarks.

**Some minor points should be addressed:**

1) Legend to figures 1 and 4 should give more information on the statistical analysis (test utilized, number of experiments performed, etc.)

Response:

We have added the statistical information into the corresponding figure legend. The revisions were listed below.

“Fig. 1 Growth rate of muscle fiber diameter (A) and weight (B) during the postnatal muscle development stage. Three replicates were used when performed the statistical analysis between different development stages. “\*” indicates significant difference (Student’s *t*-test, *p* < 0.05).”

“Fig. 4 Validation of the sequencing data using real-time PCR for nine representative DE miRNAs. The SPSS software was used to calculate the Pearson correlation coefficient (*r*) and corresponding significance value (*P*).”

2) The sentence in introduction (line 93): “our study will contribute to genetic improvement of meat quality" is not justified by the data. The authors should either explain it or remove it.

Response:

We have remove this inappropriate statement.

3) The authors did not quote the paper from Qin et al, 2013 (Integrative Analysis of Porcine microRNAome during Skeletal Muscle Development by Qin L, Chen Y, Liu X, Ye S, Yu K, et al. (2013) Integrative Analysis of Porcine microRNAome during Skeletal Muscle Development. PLoS ONE 8(9): e72418. doi: 10.1371/journal.pone.0072418), describing a very similar experimental approach. Similarities and differences should be described and commented.

Response:

Thank you for this suggestion. We have read throughout this paper and found it a valuable resource to improve our findings. We have also added some comments about the similarities and differences from their results. The corresponding revisions in the main text were listed below.

“In our study, miR-378 showed the highest expression level in the E90 stage. Though Hou et al. found that the expression level of miR-378 increased at 65 and 90 dpc and peaked at postnatal day 0, these results both suggested that miRNA-378 was a new candidate miRNA for myogenesis in pigs (Hou et al. 2012; Qin et al. 2013). These results supports the idea that it has an important role in fetal muscle development. Another miRNA, miR-148a, belong to the top ten expressed miRNAs only in E90 period, which was in line with previous finding that the average abundance of this miRNA before birth was eight times higher than that in postnatal (Qin et al. 2013). ”

“Previous study performed by Qin et al. indicated that most of the highly expressed miRNAs in porcine skeletal muscle such as miR-1 and miR-133 will be more functional. Besides the miR-1 and miR133a that we found highly expressed in all the stages (McCarthy & Esser 2007), miR-26a also showed abundant expression (Huang et al. 2008).”

**Comments for the author**

No comments

**Reviewer 2**

**Basic reporting**

The work of Mai et al. aimed to obtain the microRNA profile of skeletal muscle during porcine development, extending the results of previous analysis, performed mostly during prenatal development, to middle-aged adult muscle when muscle growth is terminated. By including samples from middle aged pigs, authors intended also to identify miRNAs associated with muscle loss. The results of the work might advance the knowledge about miRNAs involved in the regulation of adult myogenesis in pigs, an issue that is still unexplored, therefore they meet the criteria to be published, upon some minor revisions. The purposes of the authors are clear in the introduction, I noticed that the sentence included in lines 74-77 is missing one verb (probably "increase").

Response:

We are so sorry for this carelessness and have added the verb “increased” into the sentence.

1) In the diagrams of Fig. S1C and D, the p values are not indicated while described in the text: "we found that muscle fiber diameter and body weight were significantly increased (P < 0.001, one-way analysis of variance) from 0 d to 7 180 y".

Response:

We have added the statistical label into the figure S1.

2) Given that one of the purposes of the authors is to identify miRNAs that are involved in the control of muscle homeostasis, in the "results section" describing the miRNA transcriptome profile, I would suggest to include references that describe the involvement of identified miRNAs in pathways that control hypertrophy and atrophy. For example for miR-27a: Huang et al. 2012, McFarlane et al. 2014. For miR-1: Kukreti et al. 2013, McCarthy & Esser 2007, Elia et al. 2009 etc.

Response:

Thank you for this constructive suggestion. According to the reviewer’s suggests, we have added some descriptions about the muscular hypertrophy and atrophy and included the suggested references. The corresponding revisions in the main text were listed below.

“These two miRNAs are most abundant during the fast-growing stage of pig muscle. Moreover, miR-27a/b, a potential regulator of myogenesis, could induce skeletal muscle hypertrophy by down-regulating myostatin, an inhibitor of myogenesis (Huang et al. 2012; Sharma et al. 2014) and miR-27b inhibition leads to more proliferation and delays the onset of differentiation (Crist et al. 2009).”

“miR-1, a muscle-specific microRNA, promotes cell apoptosis by targeting Bcl-2 (Tang et al. 2009), and could target heat shock protein 70 (HSP70) in the development of muscle atrophy (Kukreti et al. 2013).”

3) In the diagrams of figure 2 the color code is not always useful to identify the associated miRNAs (for example I had difficulties to identify miR-378): I would suggest to enlarge the squares that describe the color code.

Response:

Thanks for this suggestion. We have changed the color code of miR-378 to a more light color to make the readers easier to follow our results.

4) Authors should compare their results to those obtained in mice (Hamrick et al. 2010) and pigs (Hou et al. 2012, Huang et al 2008) and discuss overlaps and potential contradictions.

Response:

We agree with you that it will be of significance that comparing our results to the references mentioned above. Therefore, we added some discussion as following.

“Though Hou et al. found that the expression level of miR-378 increased at 65 and 90 dpc and peaked at postnatal day 0, these results both suggested that miRNA-378 was a new candidate miRNA for myogenesis in pigs (Hou et al. 2012; Qin et al. 2013). These results supports the idea that it has an important role in fetal muscle development. Another miRNA, miR-148a, belong to the top ten expressed miRNAs only in E90 period, which was in line with previous finding that the average abundance of this miRNA before birth was eight times higher than that in postnatal (Qin et al. 2013).”

“Previous study performed by Qin et al. indicated that most of the highly expressed miRNAs in porcine skeletal muscle such as miR-1 and miR-133 will be more functional. Besides the miR-1 and miR133a that we found highly expressed in all the stages (McCarthy & Esser 2007), miR-26a also showed abundant expression (Huang et al. 2008).”

5) Given the importance of the middle-aged muscle for the purposes of the authors, I would include the validation of some Differentially Expressed miRNAs that are up-regulated in the muscle obtained from 7 years old pigs, indeed all the 9 miRNAs analyzed in figure 4 are down-regulated in this stage.

Response:

Thank you for your suggestion. The validation of DE miRNAs that are up-regulated in middle-aged pigs will, to some extent, improve our manuscript. However, we are regret that no sample now could be used to do such validation. We are sorry for that. We believe that this point is important and will be validated and investigated in our further study.

6) In figure 5 are reported the expression patterns of differentially regulated miRNAs and I think that, at least for pattern 4, the specific miRNAs should be disclosed to the readers, given that they include miRNAs upregulated in 7 years-old muscles and are involved in the regulation of pathways that control muscle homeostasis, an issue of particular interest in this work.

Response:

We agree with the review’s opinion that the miRNAs in the four significant patterns in figure 5 are quite important because of their possible roles in regulation of pathway that control muscle homeostasis. Therefore, we provided supplementary table 7 named “Table S7 Significant miRNA expression patterns during porcine muscle development” that included the miRNA list for pattern 1 to 4.

**Experimental design**

No comments.

**Validity of the findings**

1) Data are robust and findings are interesting, nevertheless I think that it will be important to indicate the miRNAs that are upregulated in middle-aged muscles (pattern 4 of Figure 5).

Response:

We agree with the reviewer that the up-regulated miRNAs in middle-aged muscles are particularly important. Therefore, we added some discussion about these miRNAs. The added discussion were listed below.

“Particularly, miR-1 showed significantly increasing expression level in 180d and 7y stages (Fig. 5A and Table S7). By using both C2C12 myotubes and dex-induced muscular atrophy mouse models, Kukreti et al. indicated that miR-1 is a muscle-specific microRNA and has a role in promoting muscle atrophy (Kukreti et al. 2013). On the other hand, McCarthy et al. also revealed that miR-1 decreased during mouse skeletal muscle hypertrophy (McCarthy & Esser 2007). Our result was consistent with the findings in mouse. Hence, miR-1 could be a potential modulator in regulating porcine postnatal skeletal muscle development.”

2) In the conclusion section, authors state that "enhanced levels of apoptosis-related miRNAs in middle-aged pigs revealed possible elevated muscle atrophy during this period" (lanes 328-329), but this statement is not supported by the findings, indeed the average cross-sectional area of the fibers (Fig. S1) do not indicate atrophy.

Response:

The cross-sectional area of the fibers for 180 d and 7 y indeed showed no significant difference. We speculated that it was due to the relatively fewer postnatal/adult time points used in this study. Only two adult periods representing the young (180d) and middle-aged pigs (7 y) were used in our study, and pigs with more consecutive ages are needed in further study to uncover the underlying mechanism of refined postnatal pig skeletal muscle development. We have added some limitations and prospects into the conclusions.