Reviewer's report

Title: Expression profiles of muscle disease-associated genes and their isoforms during differentiation of cultured human skeletal muscle cells

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Reviewer: Satoru Noguchi

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Abdul-Hussein et al. reports the expression of transcripts of sarcomeric protein and three myogenic factor genes, and immunostaining of their protein products in human skeletal muscle cells at proliferation and differentiation stages. The results have been reported on several papers on mouse, rat, chicken...including human, previously and had no novelty. There are also a big concern in study design and methods. In background, the author introduce “the formation of contractile myofibrils requires the stepwise onset of expression of muscle specific proteins.....”, however actually they only analyzed the expression of the proteins at two different points. How do they describe the stepwise progression of the formation of contractile myofibrils? They also claim functional importance of the proteins only based on their expression. This issue will be much beyond their findings.

Major Compulsory Revisions

1. They showed the expression of transcript on gel image of PCR products. However, points are not “present “or “absent”, they should measure the amounts of transcript.

2. They presented the sequence data of PCR products. The natures of the products are not clear, because many alternative splicing forms are expressed from sarcomeric protein genes along the myogenic differentiation. They should provide the information of products, which they analyzed. The sequence data should be omitted.

3. The natures of the culture cells are also difficult to be evaluated. They showed that a few cells expressed the some sarcomeric proteins (MyHC isoforms and myogenin) at proliferating stage. This might be related to the changes of the proliferating properties or misregulation of the gene expression in mononucleated myoblasts in culture. The authors mentioned they used 5 baichs of culture cells. How many proliferating cells are expressing the MyHC isoforms/myogenin in each batch? Does this population differ from culture to culture, passage...?

4. The characters of the antibodies used in this study are not cleared. They should provide the western blot data.

5. The differentiation rate is not clear, because there are so different in the number of myotubes among images. They should provide phase contrast images. In method section, the authors said they incubated the cells, for 6, 16, 24 days, however, tln results, the myotubes were only cultured for 6 and 5 days.
6. Immunostaining and immunofluorescence data are redundant.

7. The author claimed the importance of the proteins by their expression. If they will claim that, they need the overexpression and gene knockdown experiments.

8. The authors said "the maturation of the differentiated cells" by titin immunofluorescence. However, the striation patterns could not be recognized on the staining of the other sarcomeric proteins than titin. It is difficult to say “mature striated pattern of the myofibrils in vitro within few days.

9. Tropomyosin and troponin I/T/C work as forming a complex. The expression will not always indicate a functional complex formation.

10. Timing of myogenic differentiation in culture would not be related to the time of occurring diseases even in congenital disorders. The failure of onset of myogenesis will result in the embryonic lethality.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests