Author's response to reviews

Title: Entrapment Neuropathy Results in Different MicroRNAs Expression Patterns from Denervation Injury in Rats

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Author's response to reviews: see over
Dear reviewer Gulcan Gurer:

Thank you for your time, effort and professional comments in regard to our manuscript entitled “Entrapment Neuropathy Results in Different MicroRNA Expression Patterns from Denervation Injury in Rats” to *BMC musculoskeletal disorders*.

Under your kind suggestion, this article had been paid for English correction; we hope that will satisfy your standard. If required, we are very delighted to make further change or revision.

Thank you very much

Ching-Hua Hsieh, M.D., PhD
Department of Plastic and Reconstructive Surgery,
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Dear reviewer Xing Zhao:

Thank you for your time, effort and professional comments in regard to our manuscript entitled “Entrapment Neuropathy Results in Different MicroRNA Expression Patterns from Denervation Injury in Rats” to BMC musculoskeletal disorders. I have revised the document according to your suggestions and highlighted those areas in yellow color.

1. The time we chosen for the experiment is because that, via this well-established entrapment/decompression model, we had demonstrated the myopathy in a histopathological picture as atrophy of the muscle at 6 months nerve entrapment and observed some recovery of myopathy 3 months after surgical decompression (Chen CC, Jeng SF, Yang JC, Hsieh CH: Surgical decompression improves recovery of myopathy in entrapment neuropathy. J Plast Surg Assoc R.O.C. 2008;17:325-335, or you can see that from the website of Chinese Electronic Periodical Services: http://www.airiti.com/ceps/ec_en/ecjnlarticleView.aspx?jnlcattype=0&jnlptype=0&jnltype=0&jnliid=1322&issueiid=75801&atliid=1356306

We had indicated and highlighted this point in the Method/Animal surgery and tissue preparation/First paragraph.

2. Generally, the quantitative real-time RT-PCR results indicated good consistency with the results of the high-throughput microarray method in normal physiological or disease-related pathological conditions. However, it should be noted that there would be existence of some false positive and false negative targets derived from the miRNA array. In a very good study to compare the suitability of six microarray platforms and one next-generation sequencing technology to detect differential expression of miRNAs (Git A, Dvinge H, Salmon-Divon M, Osborne M, Kutter C, Hadfield J, Bertone P, Caldas C: Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. RNA. 2010;16:991-1006.), Agilent, Ambion, and Exiqon microarrays ranked highest in the rate of true differentially expressed calls with true positive/true negative rates as: Agilent, 0.90/0.86; Ambion, 0.91/0.91; Exiqon, 0.82/0.85. Our humble
experience in other miRNA array experiments also indicated around 85% true positive rate. Therefore, in current practice, with few identified targets, microarray and next-generation sequencing data are regularly validated by quantitative real-time RT-PCR; however, in a genomic miRNAs analysis, microarrays are still the best choice for a standardized genome-wide assay that is amenable to high-throughput applications. In this study, since all the four up-regulated miRNAs in the spinal segment could not be validated by the quantitative real-time RT-PCR (seemed to be much more less than 85%), therefore, we proposed our additional possible explanation as interference of the complex tissue for that in the original article, we think that in such circumstance the detection of miRNAs expression was not only influenced in the experimental groups but also in that of the control group. We had indicated and highlighted the above opinion in the revised article (Discussion/4th paragraph).

In addition, under your kind suggestion, this article had been paid for English correction; we hope the revised article and the explanation could answer well your comment and query. If required, we are very delighted to make further change or revision.

Thank you very much

Ching-Hua Hsieh, M.D., PhD
Department of Plastic and Reconstructive Surgery,
Chang Gung Memorial Hospital – Kaohsiung Medical Center, 123, Ta-Pei Road, Niao-Sung Hsiang, Kaohsiung Hsien, Taiwan
Dear reviewer John McCarthy:

Thank you for your time, effort and professional comments in regard to our manuscript entitled “Entrapment Neuropathy Results in Different MicroRNA Expression Patterns from Denervation Injury in Rats” to *BMC musculoskeletal disorders*. I have revised the document according to your suggestions and highlighted those areas in yellow color.

They are listed in the following:

1. The sentence in the Introduction starting with “In entrapment neuropathy…” had been revised, and we hope that will make it more clearly for the reader. (Background/First paragraph/Lines 11-17)

2. This entrapment/decompression model is well-established model. We did not measure the soleus muscle weight in this study; however, we had demonstrated the myopathy in a histopathological picture as atrophy of the muscle during nerve entrapment and some recovery of myopathy after surgical decompression using the histological stains with nicotine adenine dinucleotide (NADH), hematoxylin-eosin (H&E), modified Gomori trichrome and adenosine triphosphatase (ATPase) activity at pH 9.4, 4.6 and 4.3, and the quantitative measurements of cross sectional areas (CAS) of muscle fibers in a frequency histogram. Please see our previous published article (*Chen CC, Jeng SF, Yang JC, Hsieh CH: Surgical decompression improves recovery of myopathy in entrapment neuropathy. J Plast Surg Assoc R.O.C. 2008;17:325-335*), or you can see the website of Chinese Electronic Periodical Services: http://www.airiti.com/ceps/ec_en/ecjnlarticleView.aspx?jnlcattype=0&jnliptype=0 &jnltypename=0&jnlid=1322&issueiid=75801&attiid=1356306
We had indicated and highlighted this point in the Method/Animal surgery and tissue preparation/First paragraph.

3. I am very agreeable to your comment that if we could find the target genes (of the whole or each given dys-regulated miRNA) in the study will make this manuscript more meaningful. However, I have to explain our experience in this point with a little more long text. In our unpublished experiment, we want to profile the
expression of miRNAs associated with their potential target genes of the soleus muscles following 4 months denervation and re-innervation of the sciatic nerve in rats. A combined approach using computational prediction by the miRanda algorithm and the Agilent Whole Rat Genome 4×44k oligo microarray experiment was performed to indentify the potential target genes of these up-regulated miRNAs, and using GO analysis of these potential target genes into one of three ontologies: biological process, molecular function, or cellular component. The results demonstrated that the expression profiling using three replicates of array data per group revealed that there were 2262 and 777 significant down-regulated gene transcripts, if absolute expression changes were 2-fold or greater, in the denervated and re-innervated muscles, respectively. The in silico prediction discovered hundreds to thousands targets of each given miRNA. The combined approach with the computational predicted target genes and those down-regulated genes in the whole genome expression array demonstrated there were 532 and 204 potential target genes of all the up-regulated miRNAs in the denervated and re-innervated muscles respectively. We next analyzed the potential target genes to identify various cellular processes affected by denervation and re-innervation process according to Gene Ontology (GO) annotation and map into Molecular Signatures Database and compute overlaps according to GO gene sets where each GO term belongs to one of the three ontologies (biological process, molecular function, or cellular component) that showed in below three figures (grey bar is denervated tissue, black bar is re-innervation tissue, X-axis is the gene number). Therefore, we can tell that there is a different involvement of miRNAs denervated tissue, As and their potential target genes in the soleus muscle following denervation and following re-innervation of the sciatic nerve in a rat model.

However, there is two major unsolved problems in the study: First, there are still limited information that above three figures could provide; and a more important problem is that, without the loss of function experiment, some of those predicted genes may be a FALSE POSITIVE from the combined approach using computational prediction algorithm and the Agilent Whole Rat Genome 4×44k oligo microarray due to the paraphenomenonal effect. If we can use loss-of-gene approach, the identified target genes will be more solid. however, so far, there are only very few miRNA knock-out mice in the market (miR-150, miR-155,
miR-206, miR-17 to 92 cluster...etc) and the antisense oligonucleotide or LNA-antimiR is too expensive to afford in vivo experiment (10 mg = 7000 US dollars, generally in vivo effective dosage for rat = 25mg/kg, that is, for a 300-350 g rat, you have to inject the drug at the expense of around 5000 US dollars, albeit the unexpectable knock down effect).

Therefore, in the very first beginning, we had defined the study as a miRNAs expression profile study but not involving in the target genes in this stage. We hope in the future, we can have a good resolution for this. We had indicated this point in the revised article (Discussion/3rd paragraph/Lines 11-18)
4. In this study, we are not mean to only focus on miR-21. In order to use some miRNAs for representative demonstration, we have to choose those had up-regulated expression (therefore the in-situ hybridization could work), but not all the miRNA targets (it is impossible and less meaningful to do that all, Taqman and in-situ hybridization probe is expensive), and more importantly, the up-regulated miRNAs in the spinal segment could not be validated and was not suitable for further identification.

In addition, under your kind suggestion, this article had been paid for English correction; we hope the revised article and the explanation could answer well your comment and query. If required, we are very delighted to make further change or revision.

Thank you very much

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