Author's response to reviews

Title: Small non-coding RNA signature in Multiple Sclerosis patients after treatment with Interferon-beta

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To the Editor of
BMC Medical Genomics

Dear Editor,
I am sending you the answers to reviewer suggestions and the revised manuscript and figures.
Best regards,
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Answers to reviewer:
Reviewer: Mireia Guerau-de-Arellano
Reviewer's report:

Although there have been some points that have been addressed by the authors, some very important issues remain unaddressed.

Major compulsory revisions

1. Non-coding RNA cloning is typically used to identify new miRNA that have not yet been described and is not the technique of choice for profiling miRNA expression and identifying miRNA that are differentially expressed. In order to use it as a semi-quantitative technique for miRNA expression, data should be shown that proves such a relationship. Otherwise, this technique is prone to technical biases. This methodological concern has been not addressed.

   Answer: The aim of our work was to identify new small non-coding RNA by a cloning method (see for example, papers like Seungil Ro, Rui Song, Chanjae Park, et al. Cloning and expression profiling of small RNAs expressed in the mouse ovary RNA 2007 13: 2366-2380) not to profile miRNA that are differentially expressed. However, this approach allowed us to obtain a profile of sncRNAs including microRNAs, as reported in many research papers using this technique to isolate small non-coding RNAs (including microRNAs). As we show in the paper, we studied miRNAs expression by Real-Time PCR, not by the cloning strategy, therefore we removed the word “semi-quantitative” (see Results) from the manuscript to avoid confusion.

2. An assessment of RNA quality and a quality control check in the cloning would be necessary to show the procedure had no technical issues. Although some (how many remains unclear) miRNA are now listed as detected in library one, my concern that there is a technical issue remains.

   Answer: RNA samples, after the extraction by Trizol, were quality-checked by identification of 18S rRNA and 28S rRNA peaks via the Agilent 2100 Bioanalyzer platform (Agilent Technologies) (see revised Materials and Methods).

   A quality control check in the cloning has been done; in fact we used a 22-nt Small RNA Control.

   The control RNA had 5#-monophosphorylated and 3#-hydroxyl ends (5# pUUCGUUGCAGAGA-GAAAUCAC 3#), which we used to begin the control reaction at the poly(A)-tailling procedure. Beside, a tagged double-stranded DNA derived from the Small RNA Control resulted in the production of the expected ~85 bp amplicon after PCR amplification.

3. There is still a problem claiming that this study identifies a miRNA associated
with IFNbeta treatment responsiveness. Even though it is now clarified that all MS patients used in this study were IFNbeta responders, the IFNbeta treated non-responder patient cohort necessary to reach such conclusion is missing. With the current set-up, this miRNA could still just be associated with IFNbeta treatment and not treatment responsiveness, that other arm is necessary to make that conclusion.

Answer: we have checked miR-26a-5p expression in a group of non-responder IFNbeta treated patients, and we did not detect any expression change, therefore miR-26a-5p expression change is related to treatment responsiveness.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I disclose that am a PI on a grant to study miRNA in Multiple Sclerosis patients. I have no other competing interests.

Reviewer: Maurizio Taglialatela
Reviewer’s report:
Minor essential revision.
The revised version of the article by de Felice et al. is significantly improved over the first submission. Most issues I raised in my previous review have been addressed; however, I still believe that some questions remain unresolved. In particular:
1. In point 7 of my review regarding figure 5, I found the data plotted rather difficult to understand. If after 6 months of INFbeta treatment, one parameter (miR26a-5p expression) is found upregulated, and another (DGL4 expression) is downregulated, no doubt that there will be a negative correlation between the two. However, why the population stratifies in two clearly distinct, separate groups is rather strange. It would be helpful if the authors could be more explicit about what are they really plotting in Fig. 5.
Answer: in Figure 5 we plotted the miR-26a-5p expression and DLG4 expression values from
20 patients. The population looks like to stratify in two separate groups, since mainly they have similar expression values among them. I understand your point, but in the publication the graph symbols (dots) must be large and unfortunately, they overlap. I can make smaller dots to avoid confusion (see new Fig.5).

2. In general, the manuscript is rather poorly written, with very little attention to details. Some sentence are just difficult to follow. Just a few examples:
a. Abstract: ? ?ncRNAs that were associated with responder MS patients response to IFNbeta?; ???. Significantly different in the IFNbeta response?.
b. Background: ??To shed light into the mechanism ?? this sentence is incomplete.
Answer: we corrected points a and b.

3 and 6 months treatment of IFNbeta treated RRMS patients.? Again, the concept in the last sentence that has-mir-26a-5p is upregulated depends on patients response cannot be said, as there is no comparison with non-responsers (could be just a marker of disease progression!).
Answer: we changed the sentence.

c. Materials and Methods: the ethic approval statement is repeated twice.
?Patients were either treated ? at 3 or 6 months?. Most likely, patients were treated throughout the study, and blood samples were taken at 3 or 6 months.
Answer: we changed the sentence.

d. Results: fig. 3 is referred to in the text before Fig. 2, please change.
Answer: we changed.

On page12, last sentence, DLG4 is mentioned as up-regulated, whereas data show it is down-regulated.
Answer: we changed.

e. Discussion. Last sentence: DLG4 is referred to as a glutamate transporter, whereas it is not.
Answer: Since DLG4 encode for PSD95, a protein which plays a critical role in regulating NMDA receptor (a glutamate receptor) activity and its signal transduction, we changed the sentence as: glutamate-signaling related genes. DLG4 is reported as a key player in neuronal and glutamate signaling.

f. Figures. Fig. 3: label of the y axis: has-miR, please change to has-miR. Top label: beta should by in greek symbol.
Answer: we corrected both.

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.