Author's response to reviews

Title: Circulating miRNAs reflect early myocardial injury and recovery after heart transplantation

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Version: 3 Date: 4 June 2013

Author's response to reviews: see over
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Version: 3 Date: 1 June 2013
Author's response to reviews: see over
MicroRNAs (miRNAs), have been showed in human plasma and correlate with varying pathologies. In this study, the authors monitored early myocardial injury and recovery after heart transplantation by detecting level of circulating muscle-specific miR-133a, miR-133b and miR-208a. These results are interesting.

1. Besides miR-133a, miR-133b and miR-208a, there are several other muscle-specific miRNAs, such as miR-1, etc. Why only these miRNAs were used in this study?

Thanks for the reviewer’s positive and encouraging comments. In fact 6 miRNAs (miR-133a, miR-133b, miR-208a, miR-208b, miR-499 and miR-1) were included in our preliminary experiment, but unfortunately only miR-133a, miR-133b, and miR-208a showed dynamic change and correlations with perioperative parameters after heart transplantation, while the change of miR-1, miR-499, and miR-208b are different from one patient to the other, and we can’t find any regularity or correlations with perioperative parameters in these 3 miRNA, so we finally chose miR-133a, miR-133b, and miR-208a as candidate miRNAs in our experiment.

2. P2: qRT-PCR is not quantitative real-time polymerase chain reaction, but quantitative reverse transcription-polymerase chain reaction.

Thank you for these insightful and very good comments. We have already corrected this error in the method section of abstract(P3), method section in text(P8), and abbreviation(P21) as the reviewer indicated.

3. P6: “To date, no housekeeping miRNAs have been established and validated to normalize for the miRNAs content.” It’s wrong. Actually, miR-16 has been used as a reference in the detection of body fluid miRNAs (Cui L, et al. Cancer. 2013 May 1;119(9):1618-26). Should gave some discussion about this.

Thanks for the reviewer’s valuable suggestions. We gave some discussions in the text (P8) as follows:
To date, several miRNAs such as miR-16[18], or synthetic C. elegans miRNAs[3, 6, 19] have been established and validated to normalize for the miRNAs content in different body fluid. However, for detection of plasma miRNA, synthetic C. elegans
miRNAs were broadly used[3, 6, 19]. So in our experiment cel-miR-39, cel-miR-54, and cel-miR-238 were chosen and spiked in the plasma samples after combining the sample with 2×Denaturing Solution (as a mixture of 25fmol of each oligonucleotide in a 5 ul total volume)[3].

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being Published.
Thanks for the reviewer’s suggestion. Language corrections were done by our colleague Qian Zhao who has worked in the USA for several years.

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.
Reviewer's report
Title: Circulating miRNAs reflect early myocardial injury and recovery after heart transplantation
Version: 2 Date: 23 March 2013
Reviewer: Rajamiyer Venkateswaran

Reviewer's report:
Major Compulsory review:

The authors main objective of this study is to measure and monitor muscle-specific micro-RNA levels in blood and correlate with early myocardial injury and recovery after heart transplantation. Unfortunately, apart from demonstrating increased levels in blood and correlation with CTnI levels, they have failed to correlate micro-RNA levels with clinical outcomes/parameters of myocardial dysfunction.

1. It is well known that elevated donor CTnI levels correlate well with donor heart dysfunction on both haemodynamics and echocardiographic data. Does any of the micro-RNA levels correlate with donor heart function. I advise the authors to look at haemodynamic (CVP, PAWP, Cardiac index etc) and/or donor echocardiographic data to see whether any of these three micro-RNA predict dysfunction better.

Thanks for the reviewer’s valuable suggestions. We included hemodynamic data (CVP, PCWP and CO) in our analysis. We found elevated cTnI level were correlated well with CVP and CO just as you suggested. For circulating miRNA, miR-133a was correlated with CVP, miR-208a with CVP and PCWP, and miR-133b with CVP, PCWP, and CO. So miR-133b was the best one to predict graft dysfunction among of them.

We included this analysis in the manuscript
Please see “cardiac function evaluation” in method section in page 10

Cardiac function evaluation

Swan-Ganz Catheter (Edwards Lifesciences, California, USA) was implanted through external jugular vein of patients before operation. Hemodynamic parameters such as central venous pressure(CVP), pulmonary capillary wedge pressure(PCWP) and cardiac output(CO) were measured on 0 day, 1st day, 2nd day, or 3rd day postoperation (within 48-72 hours after catheterization). A score adapted from Wernovsky and his colleagues was used to quantify inotrope use for our patients[21]. Inotrope score is a reliable index of postoperative cardiac function; higher scores indicate poorer cardiac function. The score was calculated by obtaining the total amount of inotropic support the patients received at each sampling point (on 0 day, 1st day, 2nd day, and 3rd day postoperation) and then entering the data into the equation as follows: Inotrope score= Dopamine+Dobutamine+([Epinephrine+Norepinephrine+Phenylephrine]
the units of inotrope dosage used in this equation were in micrograms per kilogram per minute.

The second paragraph in Results on Page 14:
CVP, PCWP, CO, and inotrope score are indices of heart function. Higher CVP, PCWP, inotrope score and lower CO indicate worse dysfunction of heart. MiR-133a, miR-208a, miR-133b, and cTnI had positive correlations with CVP and inotrope support (Table 4). However, only miR-133b and miR-208a had positive correlations with PCWP; there were negative correlations between CO and miR-133b/cTnI (Table 4). So miR-133b was the only one that had the correlations with CVP, PCWP, CO, and inotrope score at the same time.

The second paragraph in Discussion on Page 18:
It is well-known that elevated cTnI or cTnT from donor heart is associated well with donor heart dysfunction or graft failure on hemodynamic, echocardiography or inotrope support [34-37]. In our study, cTnI levels in recipients were correlated well with CVP, CO and inotrope support at different time points after heart transplantation (Table 4). The tendency was in accordance with previous studies [34-37], although the cTnI data in our experiment were from recipients after transplantation but not from donors. We also found these 3 circulating miRNA were associated with hemodynamic indices and inotrope support. Among them, miR-133b was still the best one to predict graft dysfunction, because miR-133b was the only one that had correlations with CVP, PCWP, CO, and inotrope support. The other 2 miRNA and cTnI had correlations with only 2 or 3 of the 4 parameters (Table 4). It suggested that circulating miR-133b predict graft dysfunction better than cTnI.

Table 4. Correlations between circulating miRNA and parameters of cardiac function

<table>
<thead>
<tr>
<th></th>
<th>CVP</th>
<th>PCWP</th>
<th>CO</th>
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<tr>
<td></td>
<td>p</td>
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<td>0.117</td>
<td>0.407</td>
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Statistically significant correlations are depicted in bold.

2. The levels peak early and slowly fall over the post-operative period—Does any of the parameter correlate or predict graft dysfunction (PGD). Ventilation time and ICU stay although useful are not specific parameters of primary graft dysfunction. I advise authors to look at inotrope requirement, IABP use, iNO requirement as a marker of PGD and correlate with Micr-RNA levels.

Thanks for the reviewer’s positive and encouraging comments. As the reviewer suggested, we added inotrope requirement as a marker of PGD in our
experiment. We found that miR-133a, miR-208a, miR-133b and cTnI level correlated well with inotrope support. The correlation coefficient of miR-133b is the highest of all. So we would like to infer circulating miR-133b be proper to predict PGD. None of the 7 patients received IABP or iNO after heart transplantation. Only number 7 patient received sildenafil therapy by gastric tube because of high pulmonary vascular resistance early after operation.

The correction in manuscript
Please see “cardiac function evaluation” in method section in page 10

**Cardiac function evaluation**

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3. I note that 2 patients went on ECMO post transplantation. Is it for PGD, if so does any of the parameter predict ECMO requirement.

Thank you for these insightful and very good comments.

Indeed, 2 patients received ECMO post transplantation. They are separately number 3 and number 7 patient. These 2 patients suffered from reversible PGD early after operations. After transplantation, patients' mean arterial pressure <60mmHg; They suffered from severe metabolic acidosis and received high dose of inotrope support while hemodynamics was unstable; Inotrope scores were 29 and 51 on 0 day after operations, respectively. So ECMO was applied to these 2 patients according to indication strictly[1]. After 45.5h and 49h application of ECMO respectively, patients were getting well and met indication to withdraw ECMO[1]. Then ECMO was removed.


Minor Comments:
Authors should use common terminology- Cold ischaemic time, Warm Ischaemic time otherwise-Implantation time and these together-Total ischaemic time. I don't understand Aortic clamping time and parallell bypass time please clarify.

Thank you for these great comments.
We have rectified these terms in this manuscript. The relationship of these terms are as follows:

Total ischemia time= cold ischemia time
In our hospital, the cold ischemia time means one period from time point the donor heart was taked down to point the donor heart was transplanted into the recipient and
aortic clamp was ready to remove. There’s no perfusion into donor heart before implantation. So the donor heart was kept in ischemia condition until aorta clamp removed.

bypass time= aortic clamp time+parallel bypass time

Aortic clamp time means the time from the aorta was clamped and recipient’s heart was ready to remove to that the donor heart was transplanted into the recipient and aorta clamp was ready to remove.

Parallel bypass time means the time from aortic clamp was removed to that extracorporeal circulation was withdrawn. During this time the bypass volume was turning down and there was low level reperfusion into donor heart after ischemia. In order to investigate whether the reperfusion play a role in circulating miRNA elevation, we seperated bypass time into 2 part: aortic clamp time and parallell bypass time.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being Published

Thanks for the reviewer’s suggestion. Language corrections were done by our colleague Qian Zhao who have worked in the USA for several years.

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

Declaration of competing interests:
None