Reviewer's report

Title: Expression of circulating miR-486 and miR-150 in patients with acute myocardial infarction

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Here Zhang et al. focused on the circulating levels of two microRNAs (miRNAs or miRs) in plasma of patients with acute myocardial infarction (AMI) and showed that both miRNA levels in plasma could be used as biomarkers for AMI. Technically, the authors measured the miR-486 and miR-150 levels in plasma of patients with ST-segment elevation myocardial infarction (STEMI, n = 65) and non-ST-segment elevation myocardial infarction (NSTEMI, n = 45). Comparing those levels, the authors evaluated the circulating miR-486 and miR-150 levels in plasma of control patients (n = 110). The two miRNA levels were significantly increased in AMI patients compared with control patients, and receiver operating characteristic (ROC) curve analysis revealed that both miRNAs could be used as novel biomarker for AMI. Furthermore, the authors showed that area under curve (AUC) value, which indicates the application potency for biomarkers, became higher when the analyses of miR-486 and miR-150 were combined. Next, the authors compared the miRNA levels in patients with between STEMI and NSTEMI, and found that the both miRNA levels were significantly upregulated in NSTEMI patients compared with that in STEMI patients. The AUC values of miR-486 and miR-150 for NSTEMI patients were higher than those for STEMI patients, and the AUC value became higher when the analyses of miR-486 and miR-150 were combined. The authors concluded that these two miRNAs could be used as biomarkers for AMI, especially NSTEMI.

General comments:

This paper is relatively well written, and the purpose and conclusion can be understood easily. However, it has already reported that miR-486-3p and miR-150-3p levels are significantly increased in serum of patients with AMI, and critical readers may concern about a few questions listed below.

1. Discretionary Revisions

According to miRBase (www.mirbase.org), which is the biggest miRNA database, over 2,500 mature microRNAs are cloned in humans so far. Why did the authors focus on the miR-486 and miR-150? Did the authors conduct any screening experiments previously? Or is there any reason?

2. Major Compulsory Revisions

In Patient characteristics section of Methods, Paragraph 2: In this study, the patients with significant renal dysfunction were excluded. This exclusion criterion
is not clear. This exclusion criterion should be described accurately (e.g. eGFR or hemodialysis) because it is reported that some circulating miRNAs upregulated in AMI patients are excreted via urine (Gidlof et al. Cardiology. 2011;20:493-500).

3. Major Compulsory Revisions
From first to third section in Methods: It is better to describe the further detailed methods for the total RNA preparation from the patients and for the quantification of the miRNAs. From where was the patient’s blood taken (e.g. vein or artery)? How did the authors isolate the plasma from whole blood? How much was the starting volume of plasma to extract the total RNA? How much was the mass or volume of total RNA to reverse transcription of the miRNAs. Also, the assay ID or catalog number of the Taqman human miRNA assay kits used for quantifying the miR-486 and miR-150 should be described, because it is uncertain which mature miRNA (-5p or -3p) level was evaluated in each miRNA. I think these descriptions are very important because the detectable miRNA levels vary depending on the used method.

4. Major Compulsory Revisions
In RNA extraction section of Methods, the authors depicted that total RNA was extracted from plasma using miRNeasy Mini Kit (Qiagen). However, this kit is optimized for extraction of total RNA not from plasma or serum but from cells or tissues. From where the total RNA you extracted was derived? Did you extract the total RNA from residual cells in plasma? Also, it is known that miRNAs in plasma or serum exist in exosomes and HDL as well. Does the extracted total RNA contain exosomes-derived and HDL-derived miRNAs?

5. Major Compulsory Revisions
In qRT-PCR section of Methods: To evaluate the levels of circulating miR-486 and miR-150, U6 snRNA was used as a normalizer. While it is well established that U6 snRNA is a normalizer for evaluating miRNA levels in cells and tissues, it is not common to use U6 snRNA as an internal control for circulating miRNAs. To the best of my knowledge, spike-in method using cel-miR-39 is common in these days.

6. Minor Essential Revisions
In Figure 2, the color of diagonal line should be changed to purple. Also, it is better to change the ROC curve colors of miR-486, miR-150, and combined to blue, green, and red, respectively. Because to be consistent.

7. Major Compulsory Revisions
The authors indicated that the AUC values calculated by the combination of the two miRNAs levels were improved compared with that of miR-486 or miR-150 in Figure 2 and Figure 4. Are the improvements of AUC value between the combined data and the each miRNA statistically significant?

8. Minor Essential Revisions
In the last section of Results and Figure 4: Can you change the order of Figure 4A-4F? The order of Figure 4A-4F in the main text is different to the order of panels in Figure 4. That results in complicated. Also, it is better to change the colors of diagonal line (to purple) and ROC curves (to blue and green) to be consistent in Figure 4A, 4B, 4D, and 4E.

Additional minor issues not for publication:
1. Generally, the AUC value is indicated up to 1.0.
2. In qRT-PCR section of Methods, Page 7, line 12 (line 100): I think Taqman human microRNA assay kit is supplied from Applied Biosystems.
3. In the first section of Results, Page 9, line 4 (line 136): “EF%” should be changed to “EF”
4. In the title of fourth section of Results, Page 10, line 9 (line 163): “Pattern of expression pattern of miR-486 and miR-150”?
5. In the second paragraph of Discussion, Page 11, line 8-10 (line 184-186): miR-499, miR-1, and miR-208 are not cardiac-specific miRNAs but muscle-enriched miRNAs. Cardiac specific miRNA is miR-208a only.
6. In Table 1, the number of hyperlipidemia patients in all cohort is correct?
7. In Figure 4F, the ROC curves of miR-486 and miR-150 are correct? I assume that the colors are reversed.

Level of interest: An article of limited interest

Quality of written English: Acceptable

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.