Circulation Research

LETTER TO THE EDITOR

Response by Schulte et al to Letter Regarding Article, "Comparative Analysis of Circulating Noncoding RNAs Versus Protein Biomarkers in the Detection of Myocardial Injury"

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In Response:

We thank Dr Pinet et al[1] for their interest in our article,[2] in which they refer to our finding that patients' quantifiable plasma levels of long noncoding RNA (lncRNA) LIPCAR do not change within 24 hours of the substantial acute myocardial injury caused by transcoronary ablation of septal hypertrophy. The authors challenge our interpretation that circulating LIPCAR levels are not of primary cardiac origin. We appreciate the opportunity to further discuss this matter.

In their original article, Thum et al stated that 'recently, it was found that mitochondrial lncRNAs contribute to >70% of the total lncRNA pool of the human left ventricle, suggesting that a good proportion of mitochondrial lncRNAs detected in circulation might come from the heart'.[3] We thought it would be important to test this interesting hypothesis. Given our findings of absent lncRNA plasma elevation up to 24 hours after transcoronary ablation of septal hypertrophy, it seems unlikely that cardiac tissue is a major source of lncRNAs. Furthermore, Thum et al demonstrated a downregulation of LIPCAR 5 days after myocardial infarction but an upregulation after 1, 3 months, and 1 year in patients with cardiac remodeling. LIPCAR levels were even higher in chronic HF than in patients with ongoing left ventricular remodeling 1 year after myocardial infarction,[3] assessing patients with reduced ejection fraction in all included cohorts. Given their underlying clinical assessments, in our opinion the authors' suggestion that LIPCAR is of cardiac origin is counter-intuitive. An increase in plasma levels of LIPCAR as a cardiac-derived molecule in a subsequent chronic state seems highly unlikely if there is no detectable release on severe myocardial injury. In a second publication, Thum et al have found that 'time since diagnosis of diabetes, plasma fasting insulin, HDL-C, and ultra-sensitive-CRP, … were significantly associated with circulating LIPCAR levels'[4] in type 2 diabetes mellitus patients, indicating a more systemic response to an underlying condition, instead of a cardiac-specific release, with which our data would concur.

We fully agree with Pinet et al[1] that determination of LIPCAR's specificity or enrichment in any cell type or organ requires a quantitative comparison of human cells from different tissues. In our experiments, we found LIPCAR plasma levels to be detectable at quantification cycle (Cq) levels around 25 to 28 cycles, which is an indicator for high abundance in the circulation—comparable with microRNAs (miR) enriched in circulating cells such as platelet-enriched miR-126 (Cq 25–27), miR-223 (Cq 22–27), or leucocyte-enriched miR-150 (Cq 26–30). These levels are not directly comparable but provide an approximation of the abundance of circulating, cell-derived noncoding RNAs. Second, in our article we offer data for LIPCAR's detection in myocardial tissue on a plasma matrix. While LIPCAR increased in a linear fashion in our spike-in experiment of myocardial tissue, the concentration of myocardium used in this experiment (0.025–0.25 ug/uL) is orders of magnitude higher than that generated in myocardial infarction, which is precisely why we undertook further validation in transcoronary ablation of septal hypertrophy. There is no doubt that LIPCAR is detectable in myocardial tissue, but it is also, unsurprisingly, detectable in other cell types. For example, we have quantified LIPCAR in isolated circulating platelets (Cq 16–18), in leukocytes (Cq 17–19), and in erythrocytes (Cq 21–23; unpublished data), confirming their high LIPCAR content. The exclusive contribution of the myocardium to plasma levels of LIPCAR in any condition is, therefore, refuted.

The fact that LIPCAR is readily detectable at baseline even in patients without myocardial injury argues for another abundant source. Human platelet turn-over is on average 100 bn platelets/d, and erythrocytes are produced at roughly 200 bn cells/d. Not all circulating cell content is shed into the circulation in vivo. However,

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even taking into account the smaller size of circulating cells compared with cardiomyocytes, cardiac injury may not be sufficient to result in a significant plasma contribution of circulating noncoding RNA contents if the transcript of interest is ubiquitously expressed and highly abundant in other cells, as our data demonstrate. In contrast, cardiac- and muscle-enriched microRNAs show low baseline levels in the circulation, but the expected rise on cardiomyocyte death alongside cardiac-specific protein biomarkers. Taken together, in our opinion, these data justify the assumption that circulating LIPCAR levels are not of primary cardiac origin. Hematopoietic cell turn-over, cell activation and hemolysis, especially during the conditions of plasma generation from whole blood, could provide alternative explanations for the high abundance of circulating mitochondrial lncRNAs—such as LIPCAR. We have now added a reference summarizing our previous findings, at least for microRNAs and YRNAs, that circulating cells can be a major source of noncoding RNAs in the circulation.[5]

Finally, we were not assessing LIPCAR as a prognostic biomarker and draw no conclusions about its usefulness in this setting.

ARTICLE INFORMATION

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Disclosures

None.

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