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Circulating Circles Predict Postoperative Atrial Fibrillation

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Atrial fibrillation (AF) is a common complication after coronary artery bypass surgery (CABG), with reported incidences between 15% and 50%. Postoperative AF (PoAF) is associated with prolonged hospital stay, hemodynamic instability, increased risk of stroke, and increased mortality.[1] To prevent PoAF and to improve treatment outcomes after CABG, routine prophylactic antiarrhythmic therapy could be administered, but because of reasons of side effects and added drug costs, it is desirable to select only high-risk patients for treatment. The most consistent risk factor for postoperative AF is advanced age, but also body mass index,[2] hyperglycemia,[3] and left atrial enlargement[4] predict post CABG-AF in some studies. Despite the identification of these risk factors, there is no widely accepted risk model to predict post-CABG AF[5] and as such, there is an urgent need to identify novel biomarkers for PoAF.

Despite their first description in the 1970s, recent advances in computational analyses of RNA sequencing have sparked great interest in a novel family of RNA molecules, the so-called circular RNAs (circRNAs).[6,7] This unusual class of RNA circles is generated by the canonical spliceosome machinery, by a backsplicing event of 2 exons, which results in a covalently closed, single-stranded RNA molecule. Because of their circular form, circRNAs are protected from ribonucleases and have greater stability than their linear counterparts.[8] In the heart alone, thousands of different circRNAs are expressed.[9] Moreover, aberrant expression of numerous circRNAs in disease, and the detection of these RNA species in the bloodstream have raised the exciting possibility that they may be used as noninvasive biomarkers. Indeed, the first studies revealed that circRNAs can be secreted in exosomes[10] and that circulating circRNAs may be used as diagnostic biomarkers for type 2 diabetes mellitus,[11] for risk stratification after myocardial infarction,[12] and for cancer diagnosis.[13]

In this issue of *the Journal of the American Heart Association (JAHA)*, Zhang and colleagues[14] demonstrated that 1 particular circRNA, hsa_circRNA_025016, derived from the transcript of the alpha-1 subunit of the L-type calcium channel (CACNA1C), holds potential as a plasma biomarker for the prediction of PoAF. Zhang and colleagues first performed circRNA microarrays in plasma of 30 off-pump CABG patients, collected 1 week before surgery. Of these 30 off-pump CABG patients, 15 patients who developed PoAF were selected and closely matched for age, sex, smoking, left atrial diameter, end-diastolic volume, and statin use with 15 patients who remained AF-free. A total of 31 circRNAs were found to be associated with PoAF, and 9 circRNAs with fold changes of >4 were selected for validation with quantitative reverse transcription-polymerase chain reaction in an independent CABG cohort of 365 patients. In this cohort, particularly hsa_circRNA_025016 showed a strong association with PoAF (odds ratio of 1.355, $P<0.0001$) and receiver operating characteristic curve analysis revealed its value as a predictor of PoAF (area under the curve 0.80). Next, a cutoff value for hsa_circRNA_025016 expression was calculated from this second cohort and used to test the predictive effect in a third cohort of 284 CABG patients. Based on this cutoff value, patients were divided into high and low hsa_circNA_025016 expression groups and it turned out that hsa_circRNA_025016 was able to predict PoAF with a sensitivity of 73.5% and a specificity of 77.8%. While these numbers indicate that hsa_circRNA_025016 holds strong potential as a predictor of PoAF after CABG, it is not the only reported biomarker with these levels of predictive accuracy. Serum levels of microRNA483-5p and mitochondrial DNA copy number in peripheral blood are 2 recent examples of markers that have also been shown to predict patients at risk of PoAF after CABG (area under the curve of 0.78 and area under the curve of 0.81, respectively).[15,16] Prospective studies, in which patients, based on their biomarker levels, are electrocardiographically monitored more closely, are treated with antiarrhythmic drugs, or will be subjected to invasive interventions, are required to show whether biomarker-guided therapy truly improves clinical outcome.

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As with all good studies, this work raises important new questions. Among them, what is the cellular source of hsa_circRNA_025106? Is it expressed in a cardiac-specific manner? And what is the underlying mechanism for the enhanced production and secretion of hsa_circRNA_025016 in plasma? hsa_circRNA_025016 is circularized from exon 3 to exon 2 of the CACNA1C transcript. CACNA1C encodes the alpha-1 subunit of the L-type calcium channel ($Ca_v1.2$), which mediates influx of calcium ions in most excitable cells, including cardiomyocytes. The vital role of $Ca_v1.2$ in the heart is illustrated by the multiple arrhythmia disorders that have been linked to mutations in CACNA1C. Gain-of-function mutation is associated with long QT syndrome type 8, also known as Timothy syndrome.[17] Loss-of-function mutations in CACNA1C are associated with short QT and Brugada syndrome, both of which are clinically associated with AF.[18] It is known that CACNA1C is subject to extensive alternative splicing, and it is conceivable that the production of hsa_circRNA_025016 may reflect the expression of different splice isoforms of the L-type calcium channel in the hearts of patients who develop PoAF. So the question is, is there is a causal link between the production of hsa_circRNA_025016, or alternative splicing of CACNA1C, and the occurrence of AF? Another question that arises: does hsa_circRNA_025016 have a biological function? Or does this circRNA represent a nonfunctional transcriptional byproduct of an alternative splicing event of CACNA1C in the heart?

Interestingly, Zhang and colleagues found a positive correlation between levels of hsa_circRNA_025016 and blood glucose concentration (r=0.27 [cohort I], r=0.30 [cohort II], both P<0.0001), which is in line with earlier studies identifying high blood glucose concentration as a risk factor for PoAF.[3] With this in mind, it is remarkable that blood glucose levels were not different in CABG patients with and without AF in the 2 validation cohorts. At this point it is unknown whether hsa_circRNA_025016 simply reflects high blood glucose levels, whether it actually contributes to these high glucose levels, or whether it marks another biological event. The mechanism proposed by Zhang and colleagues, that hsa_circRNA_025016 regulates the insulin secretion pathway by acting as a sponge for several microRNAs, remains very speculative and warrants further investigation.

There is increasing interest in testing whether RNA molecules, such as micro RNAs (miRNAs) and circRNAs, could serve as biomarkers for cardiovascular disease. In the past decade, a plethora of studies have identified miRNA biomarkers in the circulation for the diagnosis and prognosis of disease. Unfortunately, the replicability of these studies appeared rather low and thus far no miRNA-based biomarker has made its way into the clinic to assist in diagnosis or guiding therapy. There is currently no criterion standard for measuring circulating miRNAs, and the lack of consistency likely relates to variability resulting from the isolation protocol, the choice of detection-platform (ie, microarray, quantitative reverse transcription-polymerase chain reaction, RNA sequencing), different methods for internal normalization, and the low levels of RNA in plasma. Nevertheless, some studies do show replicable results.[19,20] This suggests that poor replicability might not necessarily reflect poor performance of miRNAs as biomarkers, but rather a challenge in the methods of miRNA detection. Since the same issues may underlie the accurate and reproducible detection of circRNAs in the plasma, generally accepted standardized protocols are needed before clinical use of circRNA can be considered.

The biology of circRNAs is an exciting new frontier in cardiovascular medicine. Although a great deal of additional research is needed to validate these findings in other cohorts, the present work of Zhang and colleagues highlights the promise of circRNAs as disease-specific markers.

Disclosures

None.

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