Commentary

MicroRNA-499-5p: a therapeutic target in the context of cardiovascular disease

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Cardiovascular disease remains a major cause of death worldwide. Atherosclerosis, narrowing of the arteries due to the accumulation of cholesterol in macrophages within the sub-endothelial space of the vessel wall, is the primary underlying cause of cardiovascular disease. Hypercholesterolemia is therefore an established risk factor for the development of atherosclerotic lesions and cardiovascular disease (1,2). Other major cardiovascular risk factors include smoking, high blood pressure, and the presence of type II diabetes (1,2). Unfortunately, it is still difficult to reliably predict the risk for the development of cardiovascular disease and the associated mortality in individual subjects. A clear need therefore exists for the discovery of sensitive cardiovascular disease biomarkers.

MicroRNAs constitute a large family of highly conserved non-coding RNAs that inhibit the protein expression of their target genes through modulation of the mRNA translation rate and/or stability (3). Initial findings from the oncology research field have indicated that these ~22 nucleotide long RNA species may be useful as biomarkers since several tumorous tissues display a specific change in their microRNA expression profile as compared to their related non-diseased tissues (4-7). In follow-up studies we have been able to show that the presence of cardiovascular disease in humans subjects, e.g. in unstable and stable angina pectoris patients, coincides with a change in the microRNA profile of peripheral blood mononuclear cells (PBMCs) (8). More specifically, we observed that the relative expression levels of miR-135a and miR-147 were respectively 5-fold higher and 4-fold lower in PBMC fractions from coronary artery disease patients as compared to those of unaffected controls. Our studies also revealed that the PMBC fractions of stable and unstable angina pectoris patients could be distinguished based upon the expression level of three specific microRNAs. Relative expression levels of miR-134, miR-370, and miR-198 were higher in unstable subjects that had experienced ischemic chest pain at rest within the preceding 48 h versus those that had stable effort angina of >6 months duration. It thus appears that microRNAs may be able to also serve as biomarkers of (unstable) coronary artery disease.

In the context of our previous observations, in this commentary, I would like to highlight the paper by O Sullivan *et al*. that has recently been published in the International Journal of Cardiology (9) as it provides strong novel support for the predictive power of microRNA signatures in the cardiovascular disease setting. Similar as in our study, O Sullivan *et al*. aimed to show the differential expression of microRNAs in patients with stable coronary artery disease, unstable coronary artery disease and control subjects with normal coronary angiograms. Their unstable group of patients consisted of subjects who presented with ST-Elevation Myocardial Infarction (STEMI) as diagnosed per 2013 ACCF/AHA Guidelines for the Management of

ST-Elevation Myocardial Infarction. All STEMI group inclusions therefore had presented within 12 h of chest pain onset, undergone primary percutaneous intervention (PCI), and had coronary artery occlusion confirmed by angiography. A clear improvement as compared to our studies was that O Sullivan *et al*. included such a high number of patients that they could also correct for possible interactions with known cardiovascular risk factors. Levels of miR-93-5p (increased), miR-146a-5p (decreased), miR-16-5p (increased), and miR-15a-5p (increased) were all significantly changed in plasma specimens of stable coronary artery disease patients as compared to those obtained from controls (9). However, the most striking finding of the study by O Sullivan *et al*. was that inclusion of plasma miR-499a-5p levels (increased in STEMI patients versus controls) in the prediction model significantly enhanced the sensitivity as compared to traditional risk factors alone to identify subjects suffering from myocardial infarction (9). As such, the data from O Sullivan *et al*. suggest that high plasma miR-499a-5p levels may serve as a complementary biomarker for the presence of acute coronary syndromes.

Expression profiling in pigs has suggested that miR-499-5p is highly conserved and preferentially expressed in the myocardium (10). MiR-499-5p is therefore generally regarded to be a cardio-specific microRNA in humans. Importantly, the observation by O Sullivan *et al*. that the presence of the cardiac microRNA miR-499a-5p in plasma contains high predictive power in the context of acute coronary syndromes does not stand by itself. Olivieri *et al*. showed that median circulating levels of miR-499-5p were significantly higher at admission in acute myocardial infarction patients that died within the following year as compared to those that survived the cardiovascular event (11). Furthermore, a step-wise increase in plasma miR-499-5p levels was observed as compared to healthy controls in subjects suffering from acute heart failure without evidence of acute myocardial infarction and those that did display acute non-ST elevation myocardial infarction (12,13). Moreover, studies by Gidlöf *et al*. (13) and D'Allesandra *et al*. (14) have indicated that miR-499-5p levels are transiently elevated in plasma of human subjects in response to the development of myocardial infarction. In further support of the notion that miR-499-5p levels can be used as a highly sensitive biomarker of acute cardiovascular events, Gidlöf *et al*. also observed that circulating miR-499-5p levels can reliably predict the presence of STEMI (13,14).

In agreement with the aforementioned human findings, induction of myocardial infarction induced a rapid, but transient, increase in plasma levels of miR-499-5p that peaked at 24 hours after the coronary artery occlusion in both mice and pigs (13,14). The miR-499-5p plasma profile in the murine myocardial infarction model was not mimicked by that of other microRNAs supposedly expressed specifically in cardiac muscle (14). The increase in plasma miR-499-5p levels is thus probably not due to a non-specific secretion of the microRNA from cardiac tissue, e.g. in response to myocardial infarction-associated necrosis of cardiomyocytes. Interestingly, a rapid decrease in miR-499-5p levels has been detected in response to hypoxia in cultured rat cardiomyocytes *in vitro* (15). Based upon these combined findings, one can assume that a decrease in cardiomyocyte miR-499-5p levels and concomitant rise in plasma miR-499-5p levels may therefore be a general biomarker of cardiac distress. A higher plasma level of miR-499-5p thus would associate with a higher degree of cardiac dysfunction. In accordance, athletes that have immensely challenged their heart through running a marathon also display a transient rise in circulating miR-499-5p levels (16).

The question remains as to whether the increase in plasma miR-499-5p levels is only a biomarker of cardiac dysfunction/hypoxia or if this microRNA actually plays a role in the pathogenesis of acute cardiovascular events. In their elegant study, Li *et al*. have recently addressed this issue. Overexpression of miR-499-5p in cultured cardiomyocytes lowers programmed cell death protein 4 (neoplastic transformation inhibitor; PDCD4) mRNA expression which translates into a decreased apoptosis rate, while miR-499-5p inhibition increases PCDC4 transcript and protein levels and induces cardiomyocyte apoptosis (15). In line with an inverse relation between miR-499-5p levels and cardiomyocyte death, a lower miR-499-5p expression can be found in infarcted (dying) versus non-infarcted (healthy) cardiac tissue (15). Importantly, overexpression of miR-499-5p in cardiomyocytes was able to protect the heart against myocardial infarction-associated tissue damage *in vivo*. A remarkable ~50% decrease in infarct size was noted in miR-499-5p agomir-treated mice as compared to controls (15). It has been suggested that microRNAs circulating in the plasma compartment, i.e., in membrane vesicles, can be transferred to recipient cells to facilitate cell-to-cell communication (17). When taking the *in vitro* and *in vivo* findings from Li *et al*. into account, it can be hypothesized that, under myocardial infarction conditions, hypoxic cardiomyocytes release miR-499-5p for subsequent transfer to and incorporation by unaffected cells to confer

protection against myocardial infarction-associated cellular apoptosis and tissue death.

In conclusion, the study by O Sullivan *et al*. has (1) provided substantial new support for the relevance of miR-499-5p as non-invasive biomarker of acute coronary events and (2) highlighted the general potential of circulating microRNAs as predictors of disease. The recent discovery that miR-499-5p may play a protective role in cardiomyocytes has opened up new possibilities to treat subjects at risk of developing acute cardiovascular syndromes. Thus far, phase I and II clinical trials involving microRNA-based therapies, i.e. treatment of cancer patients with a liposome-formulated mimic of the tumor suppressor miR-34 (MRX34), have not yet yielded valuable drugs (18). However, it is conceivable that therapeutic approaches aimed at increasing plasma miR-499-5p levels will be developed in the future that can be of benefit for high risk cardiovascular disease patients.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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