To:
Working Group on Coronary Pathophysiology & Microcirculation of the European Society of Cardiology
The European Heart House
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Mobility Travel Grant Report

Dear Working Group Members,

I would like to thank the Working Group on Coronary Pathophysiology & Microcirculation and the European Society of Cardiology for awarding me the Mobility Travel Grant 2017. It was a great opportunity for me to be working for one month at the Catalan Institute of Cardiovascular Sciences (ICCC), IIB-Sant Pau in Barcelona, Spain and to be a part of the great research team of Prof. Dr. Lina Badimon, a team conducting innovative research in the field of cardiovascular diseases. It has been an excellent learning experience for me in a scientific environment of the highest standard.

In the following report I will be presenting the outcome of my internship at the Institute. During my stay at the Catalan Institute of Cardiovascular Sciences I acquired new skills and learned new miRNA detection techniques useful for identification of novel diagnostic and prognostic markers in cardiovascular diseases. Also I had the possibility of making new acquaintances in the cardiovascular research field, important for future collaborations between our centers.

Cardiovascular diseases and ischemic heart disease in particular have been identified as one of the leading cause of death worldwide. Therefore there is a continuous need to explore novel diagnostic methods and therapeutic possibilities.[1]

Micro RNAs (miRNAs) are endogenously produced small RNAs that regulate gene expression at the post-transcriptional level by translational repression and/or messenger RNA (mRNA) degradation, thus affecting a variety of cell processes[2,3]. The miRNA biogenesis starts in the nucleus where miRNAs are transcribed by RNA polymerase II to primary miRNAs (pri-miRNAs). The pre-miRNAs are than processed by a complex consisting of the RNase III enzyme Drosha and its cofactor DGCR8 into precursor miRNAs (pre-miRNAs), which are than exported from the nucleus to the cytoplasm by an Exportin 5 dependent mechanism. The pre-miRNAs are further processed in the cytoplasm by another RNase III enzyme-Dicer producing a short double stranded miRNA duplex. The miRNA duplex is further unwound in a mature miRNA that will be incorporated in RISC, a complex formed by the components of the Argonaute family protein[4]. So far over 2500 unique miRNAs have been identified in the human genome.[5]

In cardiovascular diseases, miRNAs are discussed as potential specific biomarkers[2] that modulate numerous signaling pathways and cellular processes, and are involved in cell-to-cell communication.[6]

Circulating miRNAs are being quantitatively altered in various cardiovascular diseases such as atherosclerosis, coronary artery disease – acute coronary syndromes, heart failure, hypertrophy and fibrosis. They are stable despite the high extracellular RNase activity, due to their packaging in apoptotic bodies, microvesicles, and exosomes or association with lipoprotein and other RNA binding proteins.[7]

Exosomes are small (40 ~ 100 nm in diameter) membrane-derived vesicles that are secreted by multiple cell types into the extracellular space after fusion with the plasma membrane[8]. They deliver macromolecular messages (RNA and protein) that enable cell-to-cell communication and signaling[9,10].

During my internship at the Catalan Institute of Cardiovascular Sciences I have learned the technique of exosomal miRNA extraction form plasma (obtained from peripheral blood) using the ExoMir MINI kit from Bioo Scientific followed by miRNA quantification using real-time polymerase chain reaction (RT-qPCR). The ExoMir-MINI kit is designed for filtration-based capture of exosomes and other microparticles and than RNA extraction from the captured particles. The particles are captured by passing the samples over 2 syringe filters of different sizes (200 nm and 50nm) connected in series. The filters are than flushed with lysis buffer (optimized to provide maximal recovery of low-mass amounts of RNA) to lyse the captured particles and release their content. The next steps are RNA extraction and quantification by RT-qPCR.

RT-qPCR is considered to be the most powerful, sensitive, and quantitative assay for the detection of RNA expression levels. For miRNA quantification we used Megaplex Protocol from Applied Biosystems (TaqMan technology).

In the TaqMan technique the PCR reaction exploits the 5′ nuclease activity of a DNA Polymerase to cleave a TaqMan probe (containing a reporter dye at the 5′ end of the probe and a quencher dye at the 3′ end of the probe) during PCR. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. During the reaction, the enzyme cleaves the probe separating the reporter dye and the quencher dye, which results in increased fluorescence of the reporter.

For miRNA cuantification a two-step reverse transcription–polymerase chain reaction (RT-PCR) assay is needed. The first step requires a reverse transcription kit to synthesize single-stranded cDNA from RNA samples. Then, the DNA polymerase amplifies the target cDNA (cDNA can be preamplified or not before this step depending on the RNA concentration from the samples) using sequence specific TaqMan probe (primers). The presence of the target is detected in real time through cleavage of the TaqMan probe by the polymerase 5′-3′ exonuclease activity. The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and is amplified during PCR (nonspecific amplification is not detected). The fluorescence detected in the qRT-PCR is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. The results are than analysed and interpreted according to the context.

Since their discovery in 2008, circulating miRNAs have been found in blood, saliva, tears, urine and other body fluids[11] raising interest in their potential use as markers for cardiovascular diseases. Their small size, simple chemical composition, high stability in boiling water, their resistance to extreme pH changes, less complexity in comparison with proteins and a cost-effective quantification by RT-qPCR makes them excellent potential biomarkers[11] in ischemic heart disease.

Many studies have been investigating the role of miRNAs in identifying patients with acute coronary syndromes. Myocardial injury leads to appearance of cardiomyocyte specific biomarkers in the bloodstream (circulating cardiac troponins), a phenomenon helpful for early diagnosis of acute coronary syndromes (ACS). An early and accurate diagnosis is essential in order to facilitate rapid decision making and treatment and therefore improve the outcome in ACS patients. However, troponin is not entirely specific for an acute coronary event; there are many other non-cardiac conditions associated with troponin elevation.

In ACS, circulating levels of miRNAs (contained in cardiac extracellular vesicles like exosomes) are significantly elevated – as an indicator of cardiac damage – making them a promising diagnostic marker for early diagnosis of acute myocardial infarction.[12] Cardiomyocytes produce many miRNAs; among these miR-1, miR-133a/b, miR-208a-b and miR-499 are more abundantly expressed in the myocardium. In case of cardiac injury, specific miRNAs appear rapidly in the bloodstream, indicating myocardial damage.[13] In case of an ACS, miRNAs could be detected in blood earlier than cardiac troponins (under 4 hours after infarction) thus highlighting their potential advantage for the earliest diagnosis of necrosis[13,14]. Moreover, several studies showed that miRNAs detection in ACS might have better sensitivity and specificity than troponins[13].

In a recent study[15] on 332 patients with suspected ACS presenting to the emergency department, the level of miR-1, -208a -499, miR-21 and miR-146a was significantly increased in ACS patients even in those with initially negative high-sensitive troponin or symptom onset <3 hours[15]. The three miRNAs (miRNA-1, miRNA-499 and miRNA-21) were found to be strong predictors of ACS independent of other clinical variables including patient history and cardiovascular risk factors. In the statistical analysis the combination of these three miRNAs resulted in a significantly higher AUC of 0.94 than hs-troponin T (0.89)[1]. This study indicates that miRNA-1, miRNA-499 and miRNA-21 can add predictive power to the established standard for ACS diagnosis. Thi s suggests a potential value of circulating cardiac-specific miRNAs as promising biomarker for early diagnosis of ACS[16].

Ischemic heart disease can lead to serious cardiac complications therefore considerable interest has been paid to studying if there are certain miRNAs that can predict cardiac death after discharge for ACS/AMI (acute myocardial infarction) patients.[17] Studies revealed that serum miRNA-155 and miRNA-380 were three fold higher in patients with cardiac death within 1 year after discharge compared to those without this complication[1], indicating a new possible predictive marker.

Despite a recent decline of in-hospital mortality attributable to acute myocardial infarction, the incidence of ischemic heart failure in post-AMI patients has been increasing in the last years.[18] Therefore, there is a continuous search for novel biomarkers that can serve as reliable predictors of ischemic heart failure in post-AMI patients; this could help optimize the treatment and improve the outcome of these patients. In one study[18] the serum levels of the microRNAs miR-192, miR-194, and miR-34a were found to be upregulated at approximately 18 days after AMI onset in patients who survived the acute ischemic event but experienced development of HF within 1 year. Another study[19] found that miRNA-150 was able to predict LV function and remodeling after AMI, which is even superior to NT-pro BNP, the golden standard biomarker currently used in the clinical practice.[1] Low circulating levels of miR-150 were found to be associated with LV remodeling after STEMI.[1]

Another investigated aspect in patients with ischemic heart disease was the prognostic value of muscle or cardiac specific miRNAs (miRNA-1, miRNA-133a, miRNA-133b, miRNA-208a, miRNA-208b and miRNA-499)[20]. At this moment, no golden soluble biomarkers can be used to accurately predict which patients are at risk of developing ACS. Three of the miRNAs known to be linked to coronary artery disease (miR-126, miR-197, and miR-223) were found to be predictive of ACS in a prospective study with 10-year follow-up[21]. Even though further validation in required this suggest a potential role for miRNAs (miRNA-126, miRNA-223 and miRNA-197) in predicting the risk of future ACS [1].

All these data suggest that circulating miRNAs hold a great potential to improve the prognosis of ACS patients who can benefit from rapid initiation of treatment are therapy optimization.

Besides their diagnostic value, miRNAs might pave the way for personalized therapy in patients with ACS. For example one potential use is identifying who can benefit from a certain therapy like antiplatelet treatment (essential medication in ACS). This has been assessed in a study by measuring plasma miRNAs response to antiplatelet therapy at different moments in the course of the treatment. Several platelet related miRNAs (miRNA-223, miRNA-191, miRNA-126 and miRNA-150) were decreased as a result of platelet inhibition in plasma. This proves the hypothesis that circulating miRNAs can help provide a tailored effective antiplatelet therapy in ACS patients and also monitor the efficiency of specific therapy.[1,11]

According to regulation, a miRNA regulates the expression levels of multiple genes and also many miRNAs regulate a single gene expression.. However, in recent findings, microRNAs are defined as target in the pathogenesis of the disease, resulting in specific potential treatment. Targeting miRNAs with anti-miRNAs can reduce the levels of pathogenic expressed miRNAs leading to the activation of gene expression; on the contrary, the use of miRNA mimics can elevate the level of miRNAs with a beneficial effect leading to the suppression of gene expression depending on the situation.[22]

In conclusion besides the potential use of miRNAs as diagnostic and prognostic markers for early detection of cardiovascular disease, miRNA modulation could serve as promising therapeutic targets considering their key function in gene regulation. miRNAs might represent in the near future a revolution in the therapy of ischemic heart disease.

Sincerely,

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