EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Plasma Circulating Extracellular RNAs in Left Ventricular Remodeling Post-Myocardial Infarction

Kirsty M. Danielson [a,b], Ravi Shah [a], Ashish Yeri [a], Xiaojun Liu [a], Fernando Camacho Garcia [a], Michael Silverman [a], Kahraman Tanriverdi [c], Avash Das [a], Chunyang Xiao [a], Michael Jerosch-Herold [d], Bobak Heydari [e], Siddique Abbasi [f], Kendall Van Keuren-Jensen [g], Jane E. Freedman [c], Yaoyu E. Wang [h], Anthony Rosenzweig [a], Raymond Y. Kwong [d], Saumya Das [a,*]

[a] Cardiology Division and Corrigan Minehan Heart Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA
[b] Department of Surgery & Anaesthesia, University of Otago, Wellington 6242, New Zealand
[c] Division of Cardiovascular Medicine, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01655, USA
[d] Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[e] Division of Cardiology, Department of Cardiac Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada
[f] Noninvasive Cardiovascular Imaging Section, Cardiovascular Division, Department of Medicine and Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[g] Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA
[h] Dana Farber Cancer Institute Center for Computational Biology, Harvard Medical School, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 13 December 2017
Received in revised form 7 May 2018
Accepted 8 May 2018
Available online xxxx

Keywords:
Left ventricular remodeling
Myocardial infarction
microRNA
Extracellular RNA
Cardiac magnetic resonance imaging
RNA sequencing
And inflammation

ABSTRACT

Despite substantial declines in mortality following myocardial infarction (MI), subsequent left ventricular remodeling (LVRm) remains a significant long-term complication. Extracellular small non-coding RNAs (exRNAs) have been associated with cardiac inflammation and fibrosis and we hypothesized that they are associated with post-MI LVRm phenotypes. RNA sequencing of exRNAs was performed on plasma samples from patients with "beneficial" (decrease LVESVI ≥ 20%, n = 11) and "adverse" (increase LVESVI ≥ 15%, n = 11) LVRm. Selected differentially expressed exRNAs were validated by RT-qPCR (n = 331) and analyzed for their association with LVRm determined by cardiac MRI. Principal components of exRNAs were associated with LVRm phenotypes post-MI; specifically, LV mass, LV ejection fraction, LV end systolic volume index, and fibrosis. We then investigated the temporal regulation and cellular origin of exRNAs in murine and cell models and found that: 1) plasma and tissue miRNA expression was temporally regulated; 2) the majority of the miRNAs were increased acutely in tissue and at sub-acute or chronic time-points in plasma; 3) miRNA expression was cell-specific; and 4) cardiomyocytes release a subset of the identified miRNAs packaged in exosomes into culture media in response to hypoxia/reoxygenation. In conclusion, we find that plasma exRNAs are temporally regulated and are associated with measures of post-MI LVRm.

1. Introduction

There have been substantial improvements in acute myocardial infarction (MI) care in recent decades, resulting in reduced mortality rates during the acute post-MI phase. Despite this, post-MI cardiac remodeling and the subsequent development of heart failure (HF) remains a key long-term source of morbidity for this patient group (Ezekowitz et al., 2009). Left ventricular remodeling (LVRm) post-MI is a multifaceted and dynamic process that is the product of the complex interplay between infarct size, genetic and epigenetic influences on cell biology, and effects of neurohormonal antagonism (Heusch et al., 2014). Recently, there has also been growing interest in the role of inflammation in post-MI LVRm (Abbate et al., 2015). Early identification of pathological LVRm is a significant clinical challenge in the treatment of post-MI patients. Natriuretic peptides are the current gold standard biomarker for HF; however, a limitation of their use is that levels are typically only elevated once there is a change in LV function and significant remodeling has already occurred (Talwar et al., 2000). Although there is an inverse relationship between LV ejection fraction (LVEF) and likelihood of adverse remodeling, many patients with a preserved LVEF post-MI will still go on to develop adverse remodeling (Abbate et al., 2015). In fact, the majority of post-MI HF cases in the modern era occur with preserved LV function and may not be captured by alterations in natriuretic peptides (Wijk et al., 2015).

* Corresponding author at: Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA.
E-mail address: sdas@mgh.harvard.edu (S. Das).

https://doi.org/10.1016/j.ebiom.2018.05.013

Post-MI LVRm consists of distinct phases involving multiple cell types in the heart including cardiomyocytes, fibroblasts, endothelial cells, and leukocytes (Burchfield et al., 2013). The acute post-MI phase is hallmarked by cardiomyocyte death and subsequent recruitment of inflammatory cells to remove dead cells and begin the repair process. Distinct from this period is a sub-acute phase where the inflammatory response is resolved and fibroblast proliferation and secretion of extracellular matrix proteins leads to the formation of scar. Chronically, continuation of these processes and the global impact of molecular changes on cardiac function leads to what is termed "beneficial" or "adverse" LVRm (Burchfield et al., 2013). Differences in cellular response to events, timing of the resolution of inflammatory response, and the degree of fibrosis all contribute to driving LVRm down either beneficial or adverse paths (Anzai, 2013). The molecular mechanisms and markers of these stages and processes have not been fully characterized. An increased understanding of the molecular architecture of post-MI remodeling offers the opportunity to identify potential early markers of adverse LVRm and therapeutic targets to prevent the development of post-MI HF.

The past decade has seen the phenomenon of the non-coding RNA revolution, where previously disregarded RNA species have been rediscovered as critical regulators of physiological and pathological processes (Cech and Steitz, 2014). Small non-coding RNAs are short regulatory transcripts (<200 nt) including the extensively studied microRNAs (miRNA) (Rajan et al., 2014), and more recently re-discovered piwi-interacting RNA (piRNA) (Rajan et al., 2014), small nucleolar RNA (snoRNA) (Stepanov et al., 2015), tRNA fragments (Cozen et al., 2015), and yRNA fragments (Cambier et al., 2017). These RNA species can affect the expression of genes and pathways important in cardiovascular diseases (CVD) including myocardial fibrosis (Thum, 2014), coronary atherosclerosis (Aryal et al., 2014), and cardiac arrhythmias (Kim, 2013). Importantly, they are stably expressed in the circulation (extracellular RNA; exRNA) as well as in tissue, leading to potential utility as diagnostic and prognostic biomarkers of disease. Significant work has been directed to the study of plasma exRNAs in acute coronary syndrome and in coronary artery disease. However, the majority of studies have been focused on candidate plasma miRNAs (Jancovicova et al., 2017, Zile et al., 2011), rather than using an unbiased discovery approach. Hence few studies have investigated the association of other circulating non-coding RNAs with MI or LVRm in humans and the only other reported non-coding RNA species to date are long non-coding RNA (Li et al., 2017, Gao et al., 2017b, Piccoli et al., 2017).

In this study, we examine the landscape of exRNAs in human circulation by RNA sequencing (RNAseq) to determine whether changes in plasma exRNAs post-MI are associated with LVRm. Furthermore, the cellular origin and temporal regulation of candidate exRNAs is examined in animal and cell models of cardiac ischemia. This is intended as an exploratory study to identify exRNA signatures associated with LVRm, and increase our understanding of the molecular architecture and biology of LVRm. Future studies in expanded cohorts for validation are important next steps for the development of these exRNA signatures as clinical biomarkers for post-MI LVRm.

2. Materials and Methods

2.1. Patient Population (OMEGA-REMODEL Trial)

The Omega-3 Acid Ethyl Esters on LV Remodeling After Acute MI (OMEGA-REMODEL; clinicaltrials.gov identifier NCT00729430) trial was a prospective, multicenter, double-blind, placebo-controlled trial testing the effect of omega-3 fatty acid supplementation (4 g/day) for 6 months after acute MI on adverse LVRm by cardiac magnetic resonance imaging (CMR, full study report in Heydari et al., 2016). The Institutional Review Boards of the respective institutions approved all protocols involving human patients and all participants provided written informed consent. This study conformed to standards indicated by the Declaration of Helsinki. Venous blood from OMEGA-REMODEL was collected in EDTA vacutainers at the time of baseline CMR imaging (2–4 weeks post-MI), centrifuged at 2000 ×*g* for 10 min for plasma separation, and immediately stored at −80 °C. CMR phenotyping (detailed in previous work by our group; (Heydari et al., 2016)) included LV end-systolic volume index (LVESVI), LVEF, myocardial mass, infarct size, and extracellular volume fraction (ECV; a validated surrogate of myocardial interstitial expansion (Haaf et al., 2016)). Of the 331 subjects in the overall study, 238 underwent post-treatment follow-up CMR for serial comparison for LV remodeling. Baseline characteristics were compared via chi-squared (categorical) or Wilcoxon tests (continuous).

2.2. Identification of Patients with Favorable and Adverse LVRm and their Respective Propensity-Matched Controls

Reduction of LVESVI during the convalescent phase after MI has remained the most robust risk marker across early (White et al., 1987, Migrino et al., 1997) and recent clinical trials (St John Sutton et al., 2017, Doughty et al., 2004). Consistent with other acute MI clinical trials, there was a high proportion of favorable structural LVRm in patients of the randomized OMEGA-REMODEL study. We therefore defined beneficial LVRm as >20% reduction of indexed LVESV ($LVRm_{fav} = 1$) given its high specificity towards favorable post-MI HF prognosis to be consistent with the published literature (St John Sutton et al., 2017). Patients with an expansion of indexed LVESV of >15% were defined as adverse remodelers ($LVRm_{fav} = 0$). A logistic regression was then performed to identify the strongest propensity-matched patient pairs based on patient age, gender, baseline LVEF, baseline infarct size by late gadolinium enhancement, and a history of diabetes. A validated propensity score-based 5 to 1 greedy matching algorithm was used to minimize matching bias (http://www2.sas.com/proceedings/sugi26/p214-26.pdf). The 11 best-matched patient pairs were then chosen.

In addition to patients with favorable and adverse LVRm, patients with adverse electrical remodeling ($n = 10$) were identified and matched to patients without adverse electrical remodeling ($n = 10$) as described above. Adverse electrical remodeling was defined as sudden cardiac arrest or the detection of ambient ventricular arrhythmia on clinical follow-up.

2.3. RNASeq of Plasma Samples

RNAseq was performed on the 11 best-matched patient pairs with beneficial and adverse LVRm and on the 10 best-matched patients with and without adverse electrical remodeling. RNA was extracted from 1 mL plasma and libraries constructed according to our previously published methods (Danielson et al., 2017). Briefly, plasma RNA was isolated using the miRCURY RNA Isolation kit for Biofluids (Exiqon) with modified protocol and libraries were constructed and amplified from approximately 10 ng RNA using the NEBNext small RNA library prep set for Illumina (NEB). Size selection of libraries was performed by gel electrophoresis on a 10% Novex TBE gel with excision of the 140 to 160 nucleotide bands (corresponding to 21–40 nucleotide RNA fragments). Libraries were diluted to a final concentration of 2 nM, pooled, and sequenced on an Illumina HiSeq 2000 for single read 50 cycles at the Center for Cancer Computational Biology at Dana-Farber Cancer Institute.

Healthy controls were used from a different study in our laboratory and were prepared using an alternative method (Shah et al., 2017). Briefly, plasma exRNA from 26 subjects was isolated using a modified mirVana PARIS protocol (AM1556; Life Technologies) with sequential phenol–chloroform extractions (Burgos et al., 2013). RNA was concentrated using the Zymo RNA Clean & Concentrator kit (Zymo Research) and libraries were prepared using the NEXTflex Small RNA Sequencing Kit v2 by Bioo-Scientific. Pools of 15 samples were created, denatured and clustered on either a single read Illumina V3 flowcell (GD-401-3001; Illumina) or a single read rapid Illumina V2 flowcell (GD-402-

Please cite this article as: Danielson KM, et al, Plasma Circulating Extracellular RNAs in Left Ventricular Remodeling Post-Myocardial Infarction, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.05.013