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ORIGINAL ARTICLE

Myocardial and Serum Galectin-3 Expression Dynamics Marks Post-Myocardial Infarction Cardiac Remodelling

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Background Acute myocardial infarction (MI) causes significant changes in cardiac morphology and function. Galectin-3 is a novel and potentially therapeutically important mediator of cardiac remodelling. Myocardial and serum galectin-3 expression dynamics in response to the early cardiovascular outcomes after acute MI are not fully elucidated.

Methods We first performed a comprehensive longitudinal microarray analyses in mice after acute MI. We then measured the serum levels of galectin-3 in a translational porcine model of coronary microembolism-induced post-ischaemic cardiac remodelling. We validated our pre-clinical studies in humans by measuring serum galectin-3 levels of 52 patients with acute ST-elevation MI (STEMI) and 11 healthy controls. We analysed galectin-3 data in relation to the development of major adverse cardiovascular outcomes (MACO).

Results Of the 9,753 genes profiled at infarcted and remote myocardium at eight different time points, dynamic myocardial overexpression of galectin-3 mRNA was detected. In a pig model of diffuse myocardial damage and cardiac remodelling, galectin-3 localised to the areas of tissue damage and myocardial fibrosis, with proportionate increase of their serum galectin-3 expression levels. In humans, increased serum galectin-3 level was associated with in-hospital MACO.

Conclusions In this translational study, we demonstrated that galectin-3 is dynamically overexpressed in response to acute MI-induced cardiac remodelling. Elevated galectin-3 levels are associated with the development of in-hospital MACO.

Keywords Galectin-3 • Myocardial infarction • Remodelling • Microarrays • Fibrosis

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Introduction

Q5 Congestive heart failure (HF) leads to high morbidity and mortality [1]. The incidence of HF has increased recently due to better survival resulting from newly developed medical therapies and early revascularisation of acute coronary syndromes [2,3]. Early identification of the culprit mechanisms of cardiac remodelling and HF is a major research focus. Prior studies have reported elevated serum galectin-3 levels in patients with LV dysfunction resulted in poor outcomes after acute MI [4,5,6,7,8,9]. Several other population-based and clinical studies have implicated galectin-3 as a strong predictor of cardiovascular events [7–9].

After an acute MI, the myocardium hosts a complex neurohumoral and matricellular response. An abrupt tissue injury leads to loss of cardiomyocytes and surrounding microvasculature. The tissue necrosis is accompanied by a release of cellular byproducts including phospholipids. The loss of microvasculature also leads to altered local osmotic gradients leading to tissue oedema [10]. The vascular stasis and cellular chemotaxis is activated and an inflammatory response then ensues [11]. Concomitantly, a tissue reparative response is triggered, which leads to fibroblast proliferation and gradual deposition of myocardial matricellular proteins in lieu of the damaged cardiomyocytes [11–13].

Once the acute tissue injury is over, an adaptive remodelling is responsible for the maintenance of myocardial morphology and function [12,14]. The adapting ventricles often tend to dilate, whereas the lost cardiomyocytes are gradually replaced by cardiac fibroblasts and collagen fibers [15]. Overall, this dichotomy of tissue damage and repair ultimately determines the long-term outcomes related to ischaemic cardiomyopathy. A smaller infract size, early revascularisation and initiation of anti-remodelling therapy can have beneficial effects, whereas the opposite will lead to adverse remodelling and loss of cardiac function leading to HF.

Several studies have examined the post-MI course in small and large translational animal models [14,16–18]. However, given the extended course of myocardial recovery, longitudinal genome-based studies have been difficult to perform on large animal models, and determination of the pathophysiological process of myocardial tissue injury, inflammation and repair has been difficult. Therefore, we have used a preclinical small animal model for the large-scale longitudinal genomics analysis. After the completion of the initial unbiased genomic profiling, we have performed additional hypothesis-driven studies on translational porcine models of MI for the validation of initial data on galectin-3 expression in relation to the development of cardiac dysfunction. Furthermore, in support of our preclinical translational findings, we have also conducted clinical studies to validate the association between elevated serum galectin-3 levels and early development of major adverse cardiovascular outcomes (MACO) in patients with acute ST-elevation myocardial infarction (STEMI).

Methods

Murine Studies

Experimental MI

We induced MI in 24 Swiss mice (age 10–12 weeks) using our study protocol described previously [19]. Briefly, xylazine (5 mg/kg s.c.) and ketamine (1 mg/kg i.m.) anaesthetised and intubated mice underwent a ligation (6-0 prolene) of the left anterior coronary artery. After a successful closure of chest wall with 5-0 silk sutures, mice were allowed to recover at 30 °C. Sham surgeries were performed identically, except for the coronary artery ligation. Upon sacrifice, we isolated tissue RNA using an RNeasy Mini Kit (QIAGEN, Hilden, Germany). The Institutional Animal Care Committee of Maastricht University approved the procedure for care and treatment of animals. All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Microarrays

We used the Incyte mouse GEM-2 cDNA libraries with 9,753 reporter genes for a microarray analysis (Incyte Genomics, Palo Alto, CA). Duplicate hybridisations were performed on these glass chips with pooled (N=3, each group) mRNA samples obtained from acute infarct and remote myocardial regions. Since our primary question was to identify the temporal transcriptional profile following acute MI in the preclinical mouse model, we performed a comprehensive genomic profiling using multiple time points. We performed microarray analyses for a total of eight time-points so that a broad and comprehensive gene expression profiling in both infarct and remote myocardial segments could be identified. The early response to acute myocardial injury was examined at four distinct time-points (one day, two days, four days and seven days). The intermediate tissue repair and myocardial adaptive response was examined at two additional time-points (14 days and 21 days). An additional two time-points (45 days and 90 days) were chosen to identify the chronic myocardial gene expression profile in response to post-ischaemic myocardial remodelling. For maximal accuracy, we only included those reporter spots in which at least 40% of pixels displayed fluorescence more than 2.5-times local background. The protocol for data mining and validation was adopted as detailed previously [20,21].

Porcine Studies

Experimental MI

We have previously generated representative porcine models of acute MI [22,23]. The coronary microembolisation model shows multiple, heterogeneous and patchy areas of myocardial scaring, and significant loss of myocardial function. In a study subset designed to examine galectin-3 expression dynamics, we generated acute MI in six Mini-swines

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