Proteomics and microRNA profiling in cardiac fibrosis

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Abstract

Cardiac fibrosis is characterized by the deposition of extracellular matrix (ECM) and mediated by cardiac fibroblasts via the renin-angiotensin system (RAS) and transforming growth factor beta (TGFβ) signaling pathways. Recognition of TGFβ by fibroblasts leads to myofibroblast differentiation as well as induction of ECM protein expression. An imbalance between pro- and anti-fibrotic signals results in excessive ECM deposition, which is linked to arrhythmogenicity, and defective systolic and diastolic function. Since ECM proteins accumulate over time, the balance of protein synthesis and degradation is particularly important in the context of cardiac fibrosis. Additionally, microRNAs (miRNAs) target messenger RNAs (mRNAs) and control their degradation or translation into proteins, affecting both the dynamics and the composition of cardiac ECM. Proteomics and miRNA profiling are novel research tools for discovering biomarkers and therapeutic targets in disease. Bioinformatic integration of proteomics and miRNA profiling experiments will further our understanding of the balance between repair and pathological processes in cardiac fibrosis.

Keywords: cardiac fibrosis; fibroblasts; proteomics; miRNAs.

■ Heart Metab. (2012) 55:4–7

Basic Article

Introduction

Cardiac fibrosis is a common complication of cardiovascular diseases, such as cardiomyopathy and myocardial infarction. Histologically, cardiac fibrosis is characterized by extracellular matrix (ECM) deposition by cardiac fibroblasts. Initially, ECM synthesis is part of a repair process. However, an excessive fibrotic response results in perivascular and interstitial ECM deposition, which is linked to arrhythmogenicity, and impairment of both systolic and diastolic function [1]. High-throughput approaches such as proteomics and microRNA (miRNA) profiling can provide an integrated readout that will improve our understanding of the molecular basis of this disease.

Molecular mechanisms in cardiac fibrosis

Following cardiac injury, matrix metalloproteases (MMPs) are secreted by cardiac fibroblasts and by infiltrating inflammatory cells and induce extensive ECM remodeling. After this acute phase, fibroblasts deposit ECM, eventually leading to cardiac fibrosis [2]. The dysregulated response of cardiac fibroblasts is mainly mediated by the renin-angiotensin system (RAS) and

transforming growth factor beta (TGFβ) signaling pathways (Fig. 1A). The effector molecule of the RAS system is angiotensin II (Ang II), which in turn induces the expression of TGFβ [3]. TGFβ acts in an autocrine and paracrine manner leading to myofibroblast differentiation and induction of ECM protein expresssion, the hallmark of cardiac fibrosis [3,4].

TGFβ is secreted as a latent, inactive complex consisting of the mature, TGFβ protein, which is non-covalently bound to a dimer of its propeptide, the latency associated peptide (LAP). This complex is associated with certain proteins of the ECM to prevent the activation of downstream pro-fibrotic pathways [5]. For instance, small leucine-rich proteoglycans (SLRPs) (i.e., decorin or biglycan) have TGFβ-binding domains and modulate its distribution and bioavailability [6]. In contrast, other extracellular proteins such as thrombospondins or MMPs are capable

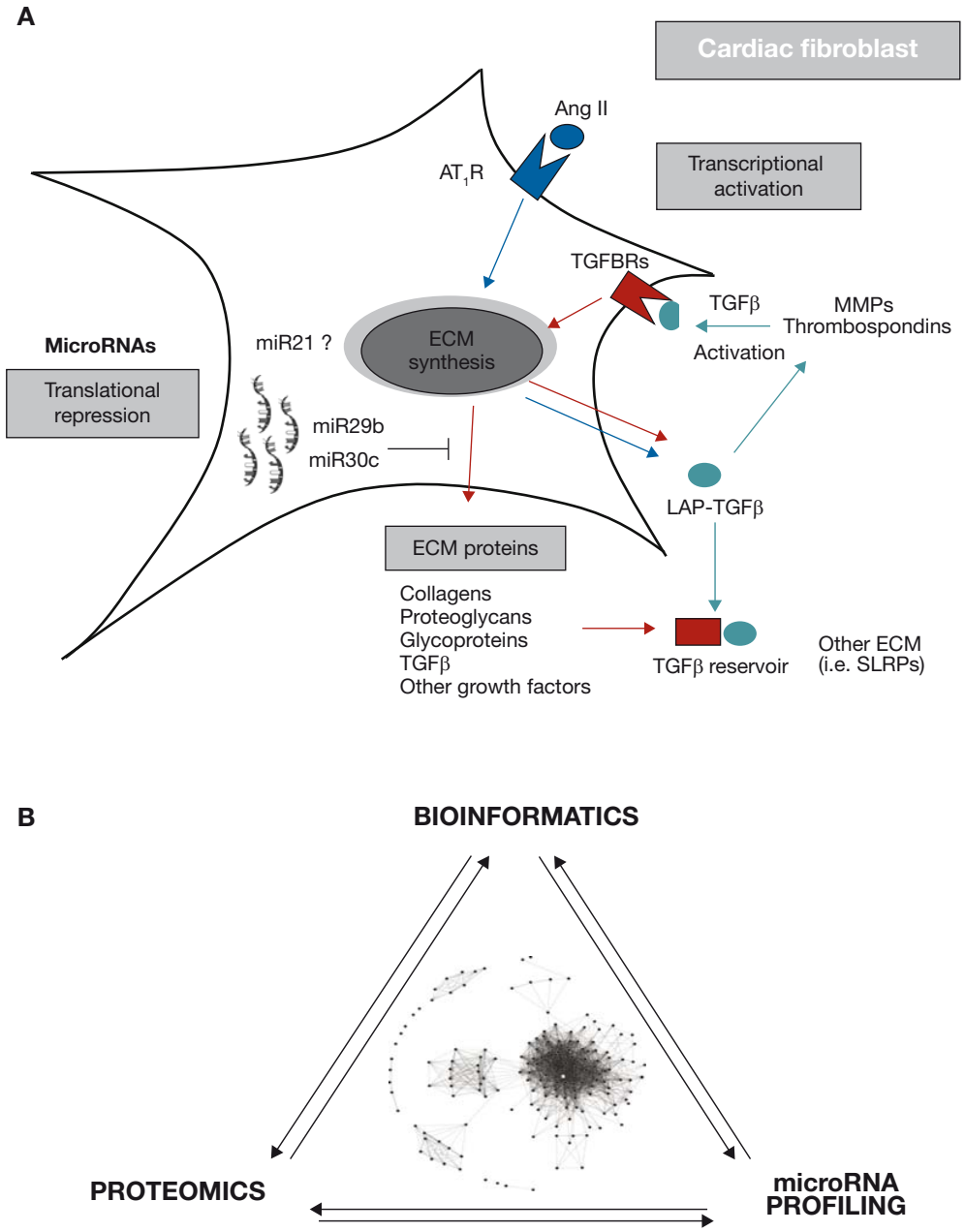

Fig. 1 ***A)*** *Regulation of cardiac fibrosis. After its induction by Ang II, TGF activates the expression of a range of ECM proteins (transcriptional activation). ECM proteins regulate TGF bioavailability. MiRNAs play a key role in controlling the translation of mRNA to proteins that are important for cardiac fibrosis (translational repression).* ***B)*** *Bioinformatics combined with integrated analysis of mRNA and proteomics profiles will be key for understanding the molecular processes underlying cardiac fibrosis.*

of activating TGFβ by cleaving the inactive LAP-TGFβ complex (Fig. 1A) [5].

Both macrophages and fibroblasts produce TGFβ. Upon ligation to its receptors (TGFBRs), TGFβ activates SMADs (mothers against decapentaplegic homolog) and other signaling pathways resulting in the expression of ECM proteins [7], MMPs and TIMPs (tissue inhibitors of metalloproteases) [2]. Besides TGFβ, the master regulator of cardiac fibrosis, molecules such as connective tissue growth factor (CTGF) are induced either by TGFβ/SMAD-dependent mechanisms or stretch-activated signaling [8] and act synergistically with TGFβ [9]. In contrast, various chemokines, cytokines and growth factors contribute to cardiac fibrosis independently of TGFβ, i.e., MCP-1 (monocyte chemoattractant protein 1) [10] or endothelin-1 [11].

Proteomics for an integrated readout of cardiac fibrosis

The proteome represents the entire set of proteins expressed in an organism, tissue, cell or subcellular fraction at a particular time point. Proteomics is an unbiased discovery approach, which is not limited to known molecules of presumed importance but enables the comprehensive assessment of protein expression in tissues and can be applied to clinical samples as well as pre-clinical models of disease. In comparison to transcriptomics (the analysis of messenger RNA - mRNA), proteomics offers certain advantages [12]. While transcript analysis can provide information on cellular activity at the time of harvest, the actual protein content depends on the balance of protein synthesis and degradation. This balance is particularly important when studying the ECM and its associated proteins because of their accumulation over time. Second, proteomics can identify changes that are not detectable at the mRNA level [13] but affect protein function in disease, including post-translational modifications (i.e., phosphorylation, oxidation, etc.) as well as proteolysis. Some proteolytic cleavage products of ECM proteins have known biological effects. Based on observed and predicted protein-protein interactions, co-expression patterns and functional similarities between proteins [14], bioinformatics can aid the identification of potential novel mediators of disease and biomarkers.

MicroRNAs regulate ECM expression

MiRNAs have recently emerged as important regulators of disease processes, including cardiac fibrosis. MiRNAs comprise a group of small non-coding RNAs that target mRNAs and control their degradation or translation into proteins. MiRNAs are encoded by introns and processed by Drosha and Dicer as reviewed elsewhere [15]. To date, almost 1000 different miRNAs have been identified in the human genome [16]. A single miRNA usually targets multiple mRNAs, often within the same biological pathway [17]. For example, miR-29b, miR-30c and miR-21 have been implicated in cardiac fibrosis (Fig. 1A). The role of miR-21, however, is controversial. In the heart, miR-21 is primarily expressed in cardiac fibroblasts and its pharmacological inhibition in adult mice was associated with attenuated cardiac fibrosis [18]. However, no effect was observed in miR-21 deficient mice [19]. In addition, miRNAs regulate glucose and lipid metabolism and insulin sensitivity [20], suggesting a role in cardiac metabolism.

Conclusion

Proteomics and miRNA profiling are novel research tools for discovering biomarkers and therapeutic targets in cardiac fibrosis. Since a single miRNA can regulate hundreds of transcripts, comprehensive proteomics profiling is the method of choice for miRNA target identification. Bioinformatic integration of proteomics and miRNA profiling (Fig. 1B) will further our understanding of the balance between repair and pathological processes in cardiac fibrosis. •

Acknowledgement Manuel Mayr is a Senior Fellow of the British Heart Foundation.

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