Identification of miRNA-497 and miRNA-27b-5p as potential diagnostic markers of cardiac fibrosis

R. Tikhomirov[1], B. Reilly-O'donnell[2], C. Lucarelli[1], S. Greco[3], G. Zacagnini[3], A. Maryam[2], L. Menicanti[3], P. Leszek[4], G. Faggian[1], P. Srivastava[2], C. Emanueli[2], F. Martelli[3], J. Gorelik[2]

[1]*University of Verona, Surgery, Dentistry, Paediatrics and Gynaecology, Verona, Italy;* [2]*Imperial College London, London, United Kingdom;* [3]*IRCCS Policlinico San Donato, San Donato Milanese, Italy;* [4]*National Institute of Cardiology, Warsaw, Poland*

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Background: Cardiac fibrosis is associated with inflammation and extracellular matrix (ECM) accumulation. A pro-fibrotic cytokine, IL11 induces cardiac fibroblasts conversion to myofibroblasts expressing α-smooth muscle actin (α-SMA) and ECM. MicroRNAs (miRNAs) are a class of small non-coding RNAs which participate in regulation of gene expression; Although mainly intracellular, miRNAs can be released into the blood stream where they can be readily detected.

Purpose: To screen miRNAs upregulated following IL11 triggered conversion of rat cardiac fibroblasts into myofibroblasts. To validate these miRNAs as potential diagnostic biomarkers of cardiac fibrosis by testing their level in blood plasma and septum of aortic valve stenosis (AVS) patients.

Methods and results: With a bioinformatical approach (Figure 1), we predicted miRNAs which can target proteins involved in TGFβ and IL-11 pathways of fibrosis progression. Of a vast number of miRNAs, we identified 7 strong candidates. After qPCR validation, we found miRNA-27b-5p and miRNA-497 to be significantly upregulated in rat cardiac fibroblasts treated by IL11 (5 ng/ul) but not TGFβ1 (100 ng/ul), values are $2^{-\Delta\Delta Ct}$: (3±1.5) and (5.2±2.2) (p-value <0.05) for miRNA-27b-5p and miRNA-497 respectively. Next, we overexpressed these two miRNAs separately in rat cardiac fibroblasts. With immunostaining we observed a (18.3±6.8)% increase in the percentage of α-SMA positive cells for miR-27b-5p and a (38.0±8.3)% increase for miR-497. Moreover, we detected with qPCR a significant up-regulation of α-SMA expression ($-\Delta\Delta Ct$ = 3.4±0.9 for miR-27b-5p; $-\Delta\Delta Ct$ = 8.2±0.7 for miR-497) in cells overexpressing miRNA27b-5p and miRNA497. Furthermore, we found that levels of both miRNA-27b-5p and miRNA-497 were significantly higher in blood plasma (p=0.0002, p=0.04) of AVS patients compared to age and sex matched control group of healthy donors (Figure 2) and heart septum (p=0.0004, p=0.04) of AVS patients compared to septum of healthy donors that could not be used for transplantation. In addition, quantification of Sirius red staining and immunohistochemistry for Col1a1 displayed significant ECM accumulation in AVS patients (p=0.04).

Conclusions: We found miRNA-497 and miRNA-27b-5p to be pro-fibrotic in rat fibroblasts. Importantly, we found both miRNAs to be up-regulated in the peripheral blood of AVS patients.

Figure 1. Identification of miRNA candidates modulating fibrosis:

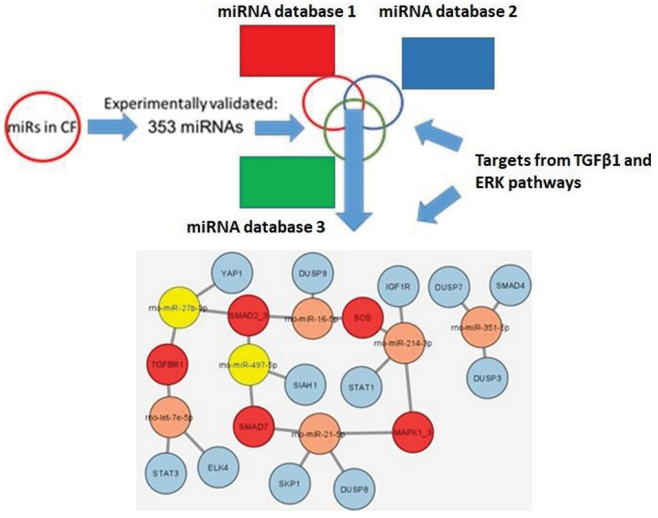

Figure 2. miRNAs levels in peripheral blood of AVS patients compared to age-sex match control group: