Scientific report in 2022

Fundamental mechanisms of post-infarction remodelling in old heart at fibroblast population level

The Inf-OLD project is focused on better understanding of the active role that cardiac fibroblasts possess in the ventricular remodelling in old animals and, as such, it aims to unveil specific signalling pathways in these cells that can be therapeutically targeted in post-infarction cardiac repair in old mice. The results obtained this year showed that miR-29a was increased in the aged body, both in the heart and other organs affected by aging-associated fibrosis (Figure 1).

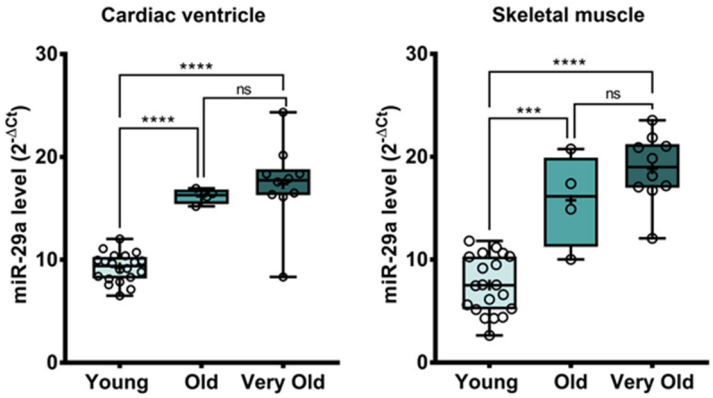


Figure 1. qRT-PCR assessment of miR-29a expression level in skeletal muscle and ventricular myocardium obtained from young (2-4 months), old (16-17 months) and very old (23-24 months) animals.

The potential impact of miR-29a increase during the aging process was investigated through bioinformatic analysis of the targets predicted by three databases used for Gene Ontology (GO)-Biological Processes enrichment analysis, which highlighted extracellular matrix (ECM) organization as the most significantly enriched GO term (Figure 2).

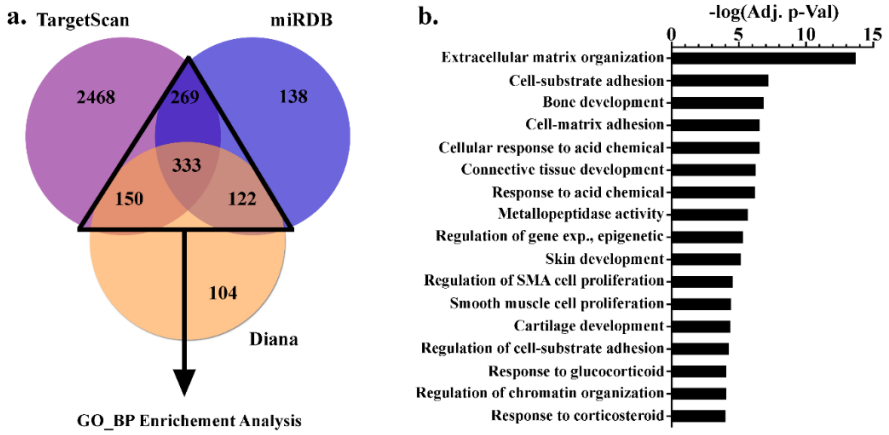


Figure 2. Identification of miR-29a targets. a) Venn diagram showing the predicted targets for miR-29a. Targets predicted by at least two databases were used in the gene ontology analysis. b) Enrichment analysis for the predicted targets of miR-29a highlighted extracellular matrix organization as the best represented biological process.

Predictive in silico analysis (Figure 3) and in vitro studies (of gene expression modulation in fibroblast cell line 3T3 and cardiac muscle cell line HL-1) identified SERPINH1, a protein with role in the stabilization of pro-collagen (by preventing incomplete folding and aggregation prior to secretion) as a potential target of miR-29a.

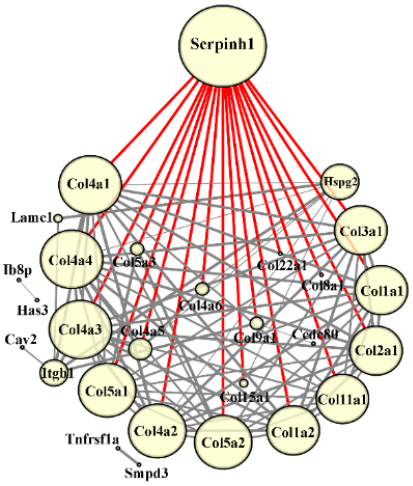
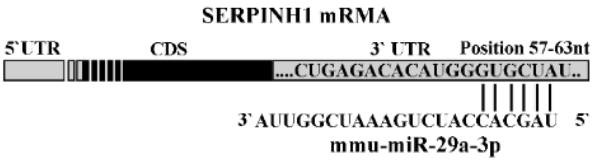


Figure 3. Identification of SERPINH1 as a miR-29a target - STRING analysis for genes involved in ECM organization.

To experimentally validate miR-29a as a modulator for SERPINH1 in the muscle cells, the gain-of-function approach was used by transient transfection with miR-29a mimic in HL-1 cardiac muscle cells. The RT-qPCR analysis showed that the overexpression of miR-29a caused a pronounced reduction of SERPINH1 (2.5-fold decrease) at the mRNA level at 1 day after transfection (Figure 4).



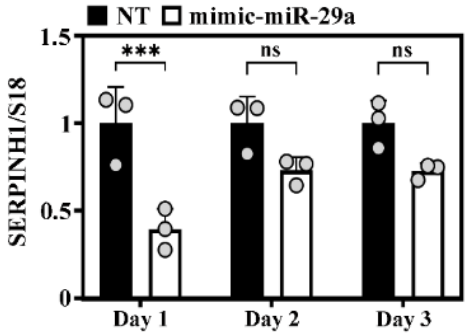


Figure 4. Representation of the 3'UTR region of the SERPINH1 gene with the predicted site for miR-29a. SERPINH1 mRNA expression in HL-1 cells after transfection with miR-29a mimic.

RT-PCT analysis of several organs harvested from young and old animals confirmed that the level of SERPINH1 was lower in the old individuals as compared to young individuals, thus suggesting that the body adapts to aging by increasing the cardiac level of miR-29a involved in reducing fibrosis through SERPINH1 repression (Figure 5).

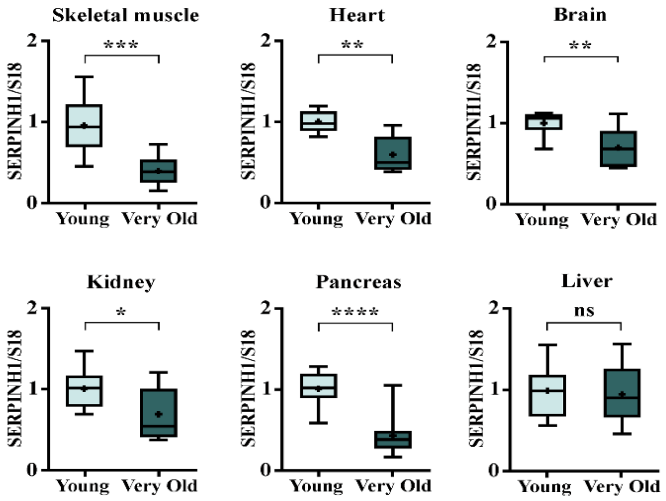


Figure 5. The expression of SERPINH1 in the skeletal muscle, the heart, the brain, the kidney, the pancreas, and the liver. For each organ, the expression is presented relative to the young group.

Furthermore, inverse correlations between the levels of miR-29a and SERPINH1 were found in multiple organs (Figure 5). In conclusion, modulation of the miR-29a - SERPINH1 axis might have therapeutic potential for reducing cardiac fibrosis in natural aging process.

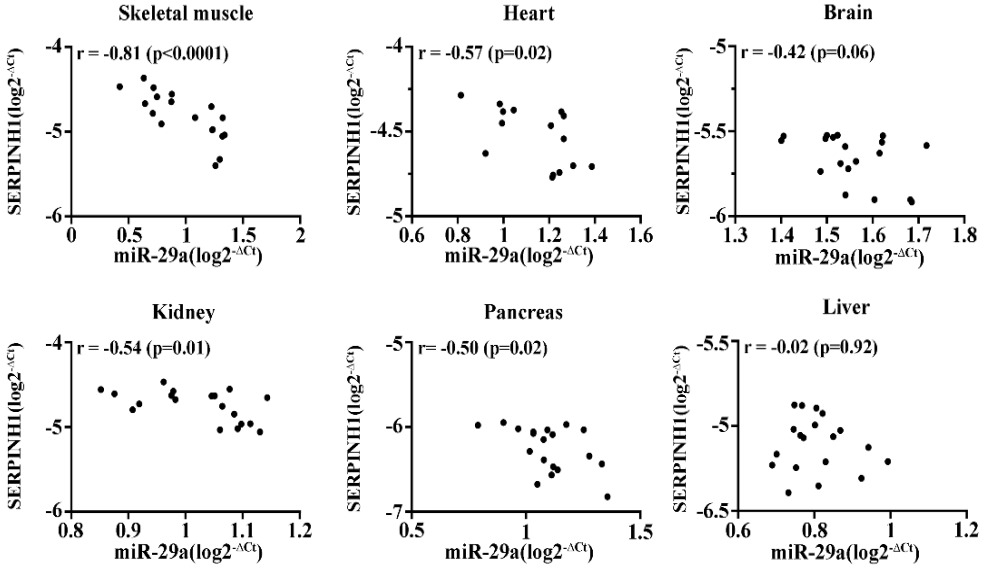


Figure 6. Correlations between SERPINH1 and miR-29a expression levels in the the skeletal muscle, the heart, the brain, the kidney, the pancreas, and the liver.

GSEA analysis of the RNA-seq results of cardiac fibroblasts isolated from young and old mouse hearts with and without myocardial infarction, showed the activation of one particular set of genes involved in epithelial-mesenchymal transition, as well as the downregulation of genes involved in cell proliferation. These irregularities were observed both in young hearts and in old hearts with myocardial infarction.

However, cell therapy seems to cause a significant reversal of both disorders. These data were validated by RT-PCR using TaqMan probes. Interestingly, these deregulations were stronger

in the area near the infarct, compared to the adjacent zones and the areas further away from the infarct. Based on the data obtained, it was hypothesized that MSC therapy stimulates the activation of fibroblasts after a heart attack and causes increased cell proliferation, without activating cardiac fibrosis. To what extent MSC influence the entire population of fibroblasts or only specific subsets is not yet known, but further studies could lead to the identification of this aspect. These studies are expected to be completed in the third phase of the project.