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PHD THESIS ABSTRACT

Cardiac cell alteration driven by the aberrant expression of ageing-associated miRNAs

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SUMMARY

Keywords: Ageing; Cardiac fibrosis; miRNA; Fibroblast; Myocardial infarction; Maladaptative remodelling; Serpinh1; Lpar2; miR-10b; Meg3-Mirg; miR-29a

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Introduction and objectives

The increase in human longevity has stimulated the interest of scientists to understand the fundamental mechanisms underlying natural aging. Ageing is a complex and still insufficiently understood phenomenon, involving a gradual decline in cellular and organ function that leads to higher incidence of chronic diseases. Numerous genetic and epigenetic modifications, as well as imbalances in biochemical processes, are tightly linked to ageing, some of which are now reorganized as hallmarks of this process (Gupta et al., 2014). According to *World Heart Report, 2023*, although the age-standardized mortality rate has decreased by one third between 1990 and 2019, cardiovascular diseases remain the leading cause of death in the world, with the number of deaths increasing by 33% over the last 30 years.

Structural and functional alterations of the heart that occur during ageing may ultimately result in heart failure (North and Sinclair, 2012). The major manifestations of cardiac ageing include fibrosis (Biernacka and Frangogiannis, 2011), chronic inflammation (Ferrucci and Fabbri, 2018), hypertrophy (Olivetti et al., 2000), and impaired angiogenesis (Lähteenvuo and Rosenzweig, 2012). When studying cardiac structure and function, most attention is traditionally given to cardiomyocytes, despite the fact that non-myocyte cells represent the majority of the heart cellular population. Among these, cardiac fibroblasts form a heterogeneous and dynamic group with essential structural and functional roles in cardiac homeostasis, both under physiological and pathological conditions. Cardiac fibroblasts account for approximately 15% of the total cell population in the adult murine myocardium, are interspersed among cardiomyocytes, and possesses a specific molecular programme that enables them to rapidly integrate and respond to extra cellular cues following injury (Tallquist and Molkentin, 2017). Activation of fibrotic signalling pathways in response to ageing related stress leads to cardiac fibrosis and hypertrophy. These are two key features of cardiac ageing that are strongly interconnected and mutually triggered by each other. Consequently, cardiac ageing is often associated with diastolic dysfunction and heart failure with preserved ejection fraction (HFpEF), which has a negative effect in the elderly population (Singam et al., 2020).

Myocardial infarction (MI) is one of the most devastating cardiovascular events. Ageing not only increases the risks of MI but also worsens post-infarction outcomes, as aged hearts exhibit impaired angiogenesis, inefficient resolution of inflammation, and reduced regenerative capacity (Olivetti et al., 1991, Dai et al., 2012). This processes ultimately drive

maladaptive myocardial remodelling. A central feature of this remodelling is cardiac fibrosis, defined by excessive deposition of extracellular matrix (ECM) proteins, particularly collagen. While fibrosis initially stabilizes the injured myocardium, excessive ECM accumulation compromises ventricular compliance, disrupts electrical conduction, and eventually contributes to heart failure (Travers et al., 2016).

Cardiac fibroblasts are the main contributors to post-MI fibrosis, but they also influence other aspects of healing, including inflammation (Theall and Alcaide, 2022) and scar maturation (Daseke et al., 2020). Thus, dysregulated activation of cardiac fibroblasts in the early stages of injury can therefore result in defective healing and worsened cardiac performance. In the later phases, the fibrotic scar-initially crucial for survival, undergoes extensive crosslinking, which increases tensile strength but compromises contractility (Deb and Ubil, 2014). These processes ultimately contribute to systolic dysfunction and chronic heart failure (Smolgovsky et al., 2021).

Increasing evidence highlights the important role of microRNAs (miRNAs) in cardiovascular pathologies associated with ageing. Some miRNAs display pro-fibrotic activity, while others exert anti-fibrotic effects in MI and fibrotic remodelling in the aged hearts. The discovery of the association between miRNAs and some diseases has fueled the interest in their therapeutic potential as a novel class of drugs. Moreover, the global success of mRNA vaccines against SARS-CoV-2 has further increased the interest for RNA-based therapies, which are now emerging as a real treatment option (Seyhan, 2024).

Based on these considerations, our **hypothesis** is that dysregulation of the miRNAs expression profile during ageing disrupts the molecular mechanisms that maintain homeostasis, and leads both to systemic functional decline and to the initiation of pathological processes such as cardiac fibrosis and maladaptive post-MI remodelling. Thus, the **aim** of this thesis was to explore the role of miRNAs as key regulators of cellular homeostasis and as potential therapeutic targets capable of counteracting pathological processes such as ECM remodelling and cardiac fibrosis. By doing so, this work contributes to the identification of innovative strategies to promote healthy ageing and prevent age-associated complications. To achieve this aim, the research carried out during my doctoral studies pursued the following **specific objectives**:

1. To identify the expression profile of miRNAs involved in the pathophysiology of the cardiovascular system in individual cardiac cell populations isolated from aged mice;

2. To assess the in vitro effects of ageing-associated miRNAs on cardiac fibroblasts, focusing on their impact on the pro-fibrotic phenotype and signalling pathways related to ageing (inflammation and tissue remodelling);
3. To determine the therapeutic potential of miRNA modulation on post-MI the maladaptive remodelling in the ageing heart.

Thesis structure

The doctoral thesis is organised into two main parts:

Part 1 - Current state of knowledge is divided into 3 main chapters. **Chapter 1**, “*The role of ageing in the onset of cardiovascular diseases*”, addresses general future of ageing, cardiac alterations associated with ageing, and finally, Age-related cardiac remodeling after ischemic heart disease. **Chapter 2**, “*The cardiac fibroblast*”, highlights aspects of the cardiac fibroblast, from its general characteristics to its role in cardiac homeostasis and fibrosis. Finally, **Chapter 3** focuses on “*The role of miRNAs in cardiac ageing and ageing-related pathologies*”. This chapter provides a synthesis of the literature regarding miRNAs nomenclature and genomic organization, their biogenesis, mechanisms of action, and their dysregulation in cardiac ageing and age-related cardiovascular diseases. At last, the chapter reviews several preclinical studies regarding the use of miRNAs in cardiac fibrosis therapy.

Part 2 - Original contributions, presents the original results obtained in the studies carried out to fulfil the objectives of the thesis. It is divided into three chapters, whose main results and conclusions are summarised below:

Chapter 1- MIRNAs INVOLVED IN NATURAL AGEING: IMPACT ON CARDIOVASCULAR DISEASES, includes the study “*MiR-29a increase in ageing may function as a compensatory mechanism against cardiac fibrosis through Serpinh1 downregulation*”. In this work, we focused on identifying the roles of four miRNAs (miR-18a, miR-21, miR-22 and miR-29a), known to be involved in cardiovascular diseases during ageing. Using molecular biology, biochemical techniques and cell culture models, we showed that cardiac ageing is associated with increased expression of miR-18a, miR-21a, miR-22 and miR-29a. To determine whether these changes are heart specific or represent a systemic hallmark of ageing, we also evaluated their expression in multiple organs collected from young and aged mice. The results showed that only miR-21a and miR-29a were

globally increased in ageing, and very interesting the expression level of miR-29a was higher than that of miR-21a (Fig. 1B).

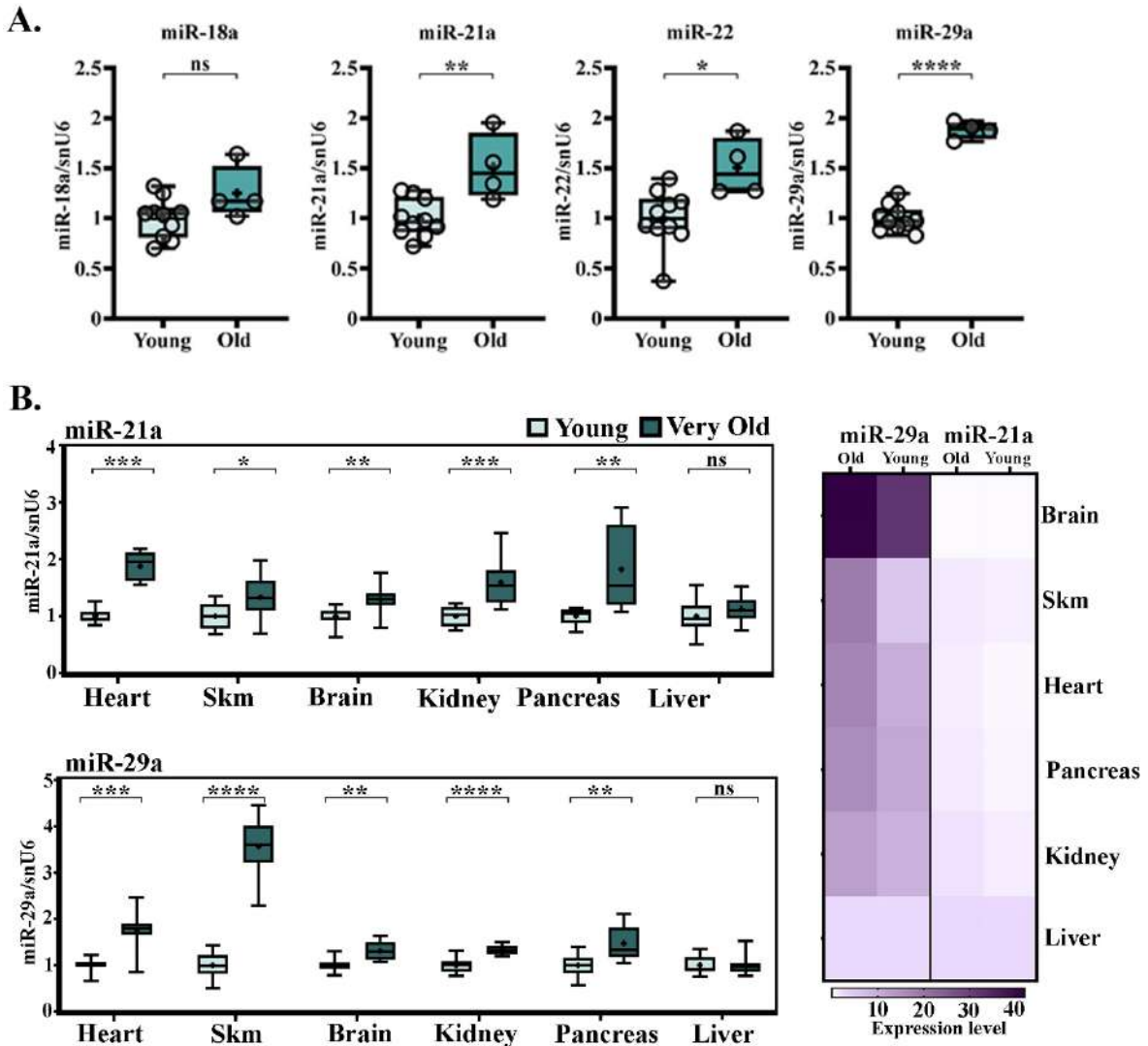


Figure 1. The evaluation of miRNAs involved in cardiac pathologies during natural ageing. (A.) Evaluation of four miRNAs in the heart collected from young and aged mice; (B.) Assessment of miR-21a and miR-29a expression levels in multiple organs harvested from young and aged mice. The heatmap shows a higher expression of miR-29a compared to miR-21 across the analysed organs. Data are presented as 2-dCt values, with snU6 used

These findings suggests that miR-29a might play a more important biological role in the ageing process, which prompted further studies focused on elucidating its function during ageing. Bioinformatic analysis revealed that miR-29a directly regulates *Extracellular matrix organization* by targeting *Serpinh1*, an essential collagen chaperone (Fig. 2A-B). Experimental validations confirmed *Serpinh1* as a direct target of miR-29a, and its

expression level was found negatively correlated with the elevated levels of miR-29a observed in aged organs (Fig. 2C).

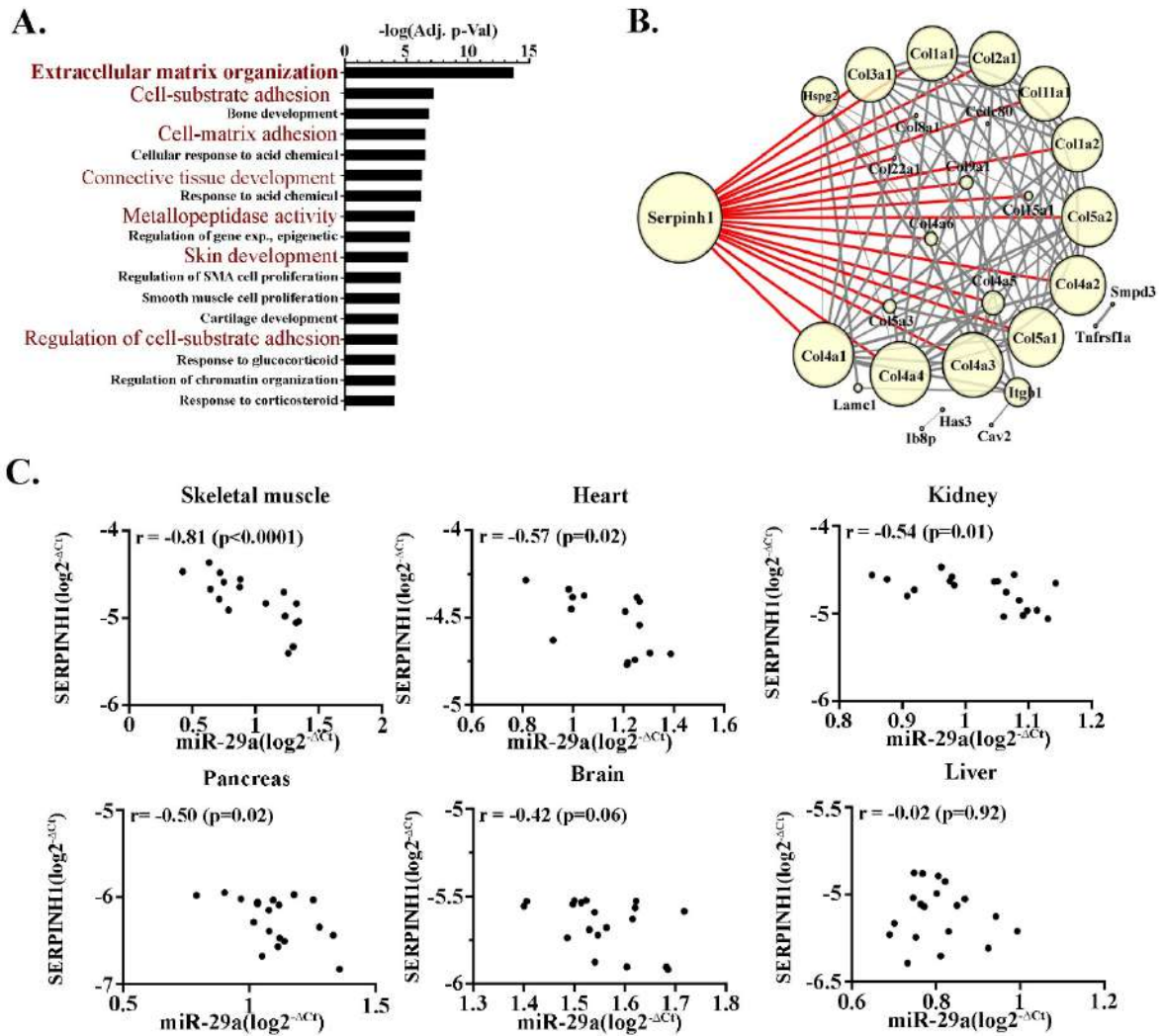


Figure 2. Identification of Serpinh1 as a target of miR-29a. (A.) Bioinformatic analysis of biological processes deregulated by miR-29a; (B.) Protein-protein interaction (PPI) analysis identifying Serpinh1 as a central player in “ECM Organisation”; (C.) Pearson correlations between Serpinh1 and miR-29a expression levels as across six analysed organs;

Finally, in vitro studies demonstrated that overexpression of miR-29a in fibroblasts and cardiac muscle cells inhibited cell proliferation and migration (Fig. 3A), and reduce the synthesis of pro-fibrotic molecules such as Collagen 1 and 3 (Fig. 3B). These findings suggest that miR-29a acts as a compensatory anti-fibrotic mechanism, capable of limiting pathological remodelling of the extracellular matrix.

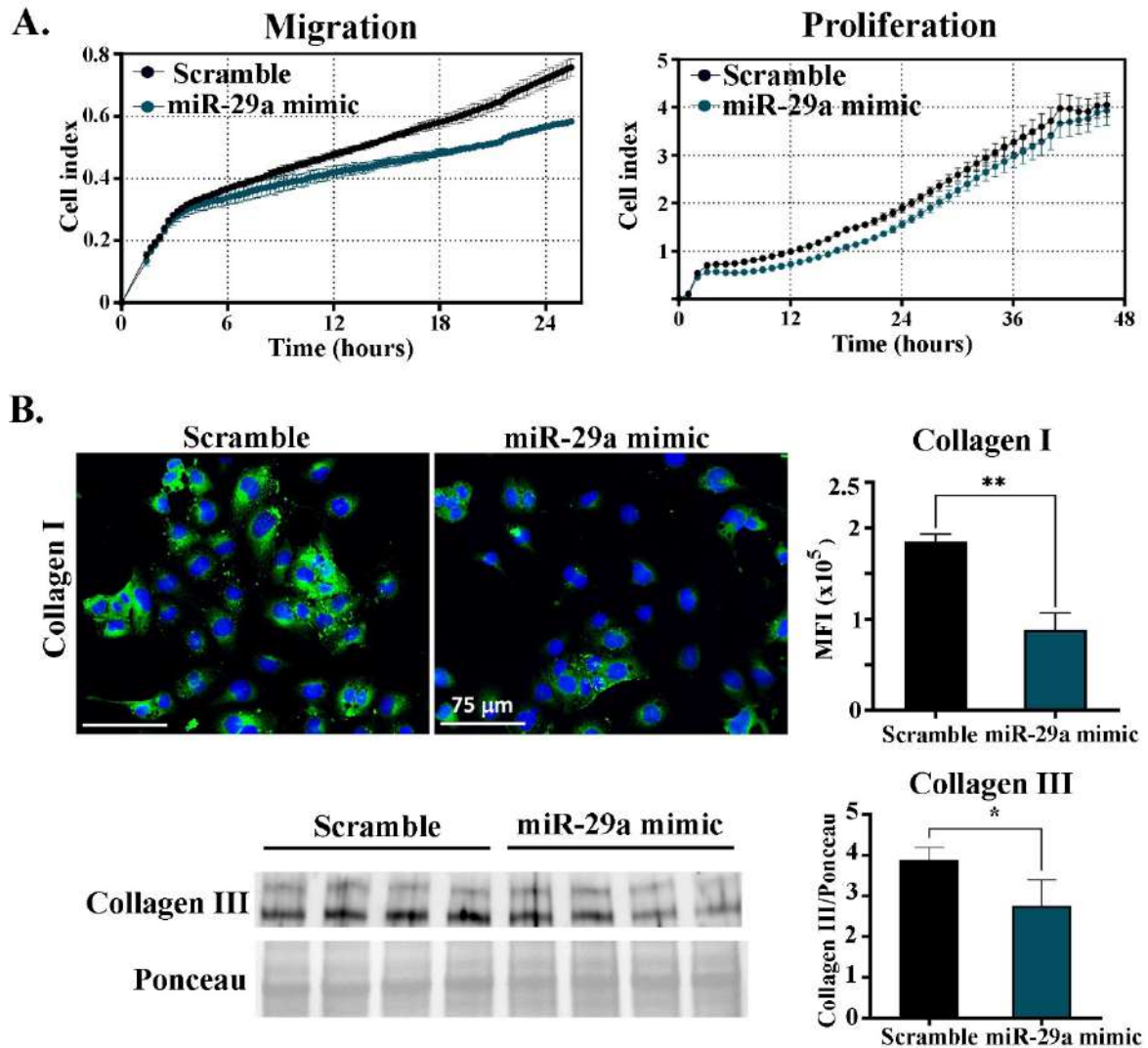


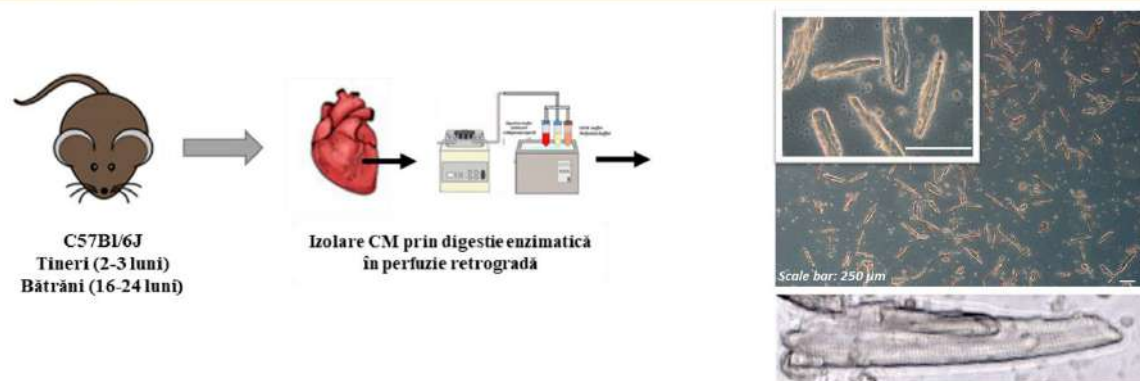
Figure 3. The antifibrotic role of miR-29a. (A.) Real-time assessment of migration and proliferation in HL-1 cells following transfection with either a miR-29a mimic or scrambled miRNA control. Data represent one representative experiment out of three performed in quadruplicate with similar results; (B.) Quantification of collagen in HL-1 cells overexpressing miR-29a.

Chapter 2-INVESTIGATION OF MIRNAS WITH REDUCED EXPRESSION IN CARDIAC FIBROBLASTS DURING NATURAL AGEING, comprises two studies:

Study 1 –“Isolation and characterization of primary murine fibroblasts and cardiomyocytes” aimed to obtain the highest number of viable and functional cardiac cells. Adult murine cardiomyocytes were isolated from young and old mice by retrograde perfusion through the aorta with a digestion buffer, which insured the uniform distribution of digestive enzymes through the coronary network, enabling an efficient digestion of

myocardial tissue. The isolated cells displayed typical rectangular morphology, being elongated and in some cases, branched (Fig. 4). To isolate cardiac fibroblasts, three enzymatic digestion protocols using different enzymes were standardized. Comparison of the three protocols revealed that fractionated digestion provided the best balance between yield, viability and cellular purity, allowing the reproducible isolation of a substantial number of cardiac fibroblasts. The fibroblasts exhibited typical mesenchymal morphology, with spindle-shaped or stellate forms.

Cardiomiocite



Fibroblaşti cardiaci

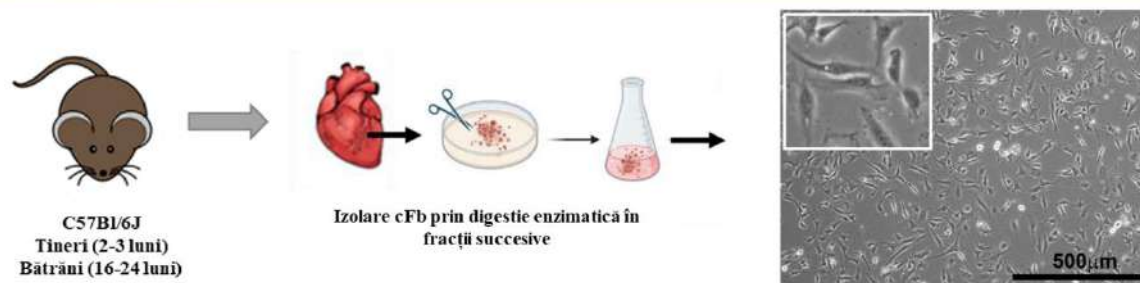


Figure 4. Isolation of cardiac cells. Schematic illustrations of the experimental design for cardiomyocytes and cardiac fibroblast isolation. Representative images of cardiomyocytes after 1h in culture and cardiac fibroblast after 3 days in culture.

Immunophenotypic characterization of cardiac fibroblasts by flow cytometry show that the cell suspension was depleted of CD45⁺ immune cells and CD31⁺ endothelial cells, while the majority of cells expressed Pdgfr-alpha, S100A4 and alpha-SMA, a well-established panel of cardiac fibroblast markers. Comparative analysis of fibroblast isolated from young and old mice reveal that the age associated phenotypic changes were moderate, but significant at the cellular level. Specifically, fibroblasts from old animals exhibited a slight increase in cell size (Fig. 5A), reorganisation of alpha-SMA (Fig. 5B) and a greater reliance

on glycolytic metabolism associated with a reduced adaptability to metabolic stress (Fig. 5C).

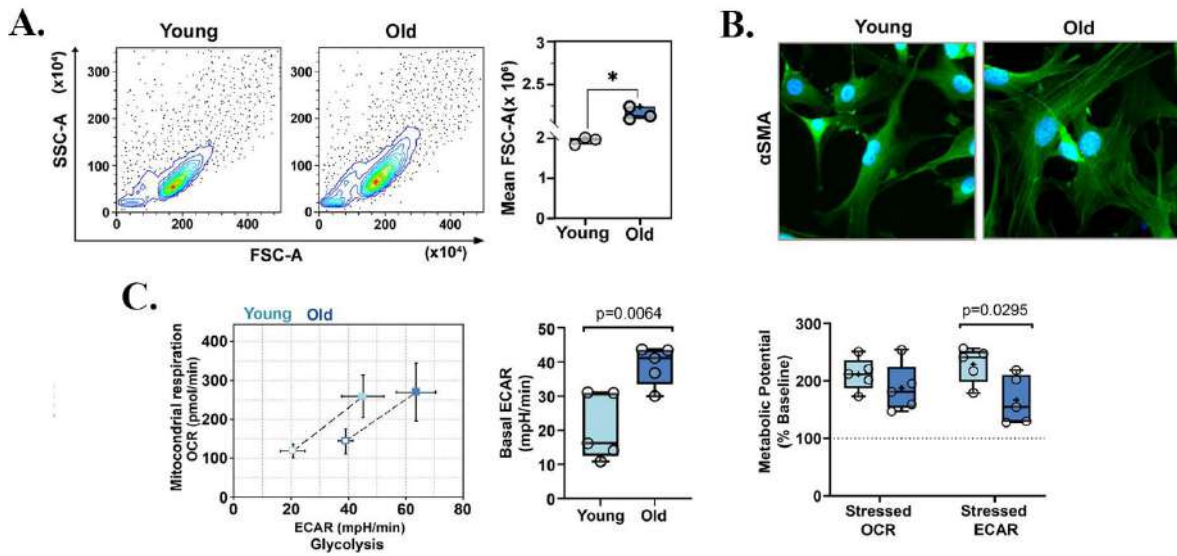


Figure 5. Differences in cardiac fibroblast derived from young and old mice. (A.) Scatter plot showing the size of cardiac fibroblast from young and old mice; (B.) Fluorescence microscopy images showing differences in the distribution pattern of alpha-SMA protein (The pictures are representative from three experiments); (C.) Metabolic differences between young and old fibroblast under basal and stress conditions.

Study 2- “MiRNAs generated from Meg3-Mirg locus are downregulated during aging”. In this study, we demonstrated that natural ageing in cardiac fibroblast was associated with moderate changes in the expression of 88 miRNA, of which 60 show significant downregulation (Fig. 6A). To identify those miRNAs with significantly altered expression that may reliably reflect ageing-associated changes we map their genomic position and the frequency of steady, upregulated and downregulated miRNAs were interrogated for each chromosome. The results reveal that among the 60 downregulated miRNAs, 30 originated from the Meg3-Mirg locus on chromosome 12 (Fig. 6B). This locus consists exclusively of non-coding RNAs, i.e. 3 long-noncoding RNAs (Meg3, Rian, Mirg), several small nucleolar RNAs (C/D snoRNAs) and 57 miRNA genes (Fig. 6C).

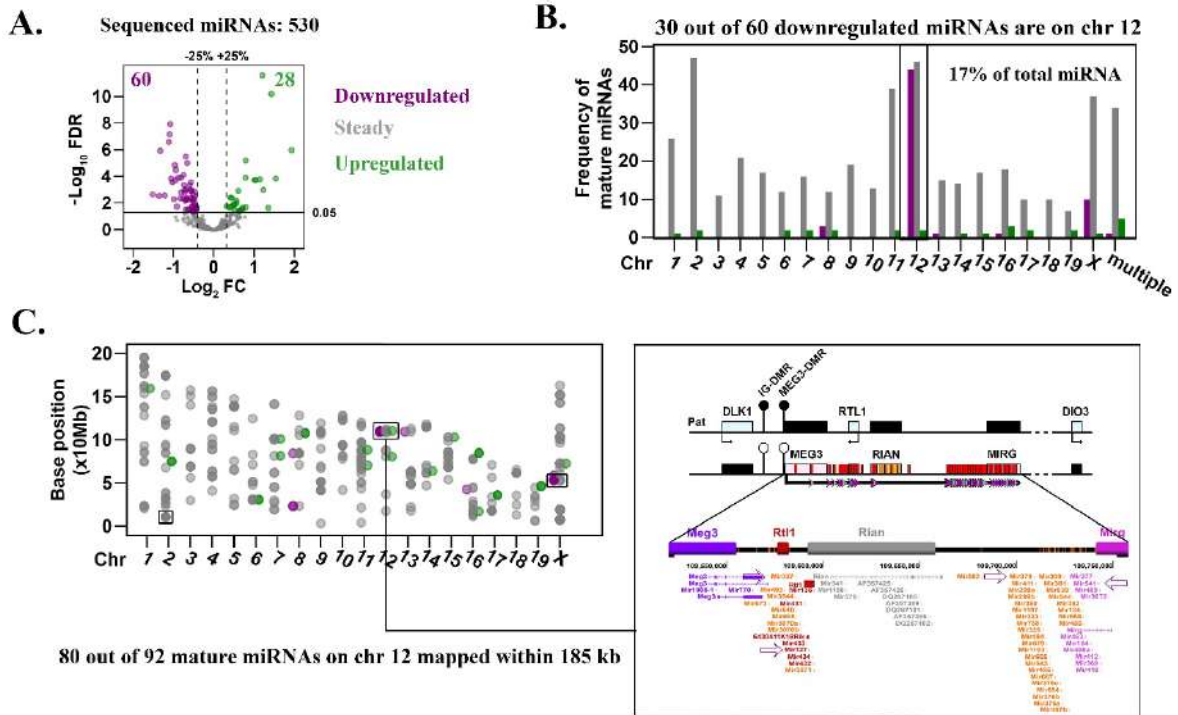


Figure 6. Identification of miRNAs in cardiac fibroblast isolated from young and old mice. (A.) Volcano plot showing the analysis of differentially expressed miRNA; (B.) The frequency of mature miRNAs mapped to each mouse chromosome or to multiple chromosomes; (C.) The genomic location of mature miRNAs represented by the position at which the corresponding gene begins (mature miRNAs with multiple genomic locations were not showed) and schematic representation of the Meg3-Mirg locus as part of the paternally imprinted Dlk1-Dio3 locus. The Meg3-Mirg locus is maternally expressed and contain exclusively non-coding RNAs, namely lncRNAs (Meg3, Rian, Mirg), several snoRNAs (orange bar) and 57 miRNA genes (red bars). Representative miRNAs from the Meg3-Mirg cluster are highlighted with arrows.

To determine whether the ageing associated downregulation of miRNAs from the Meg3-Mirg locus is a feature specific to cardiac fibroblasts or also affected other organs or cardiac cell types, we selected four mature miRNAs (miR-337-5p, miR-127-3p, miR-379-5p, miR-541-5p) as representative of the locus. These miRNAs were chosen based on their genomic positions across the cluster and their consistent expression levels. The results demonstrated that natural ageing is associated with a coordinated repression of these four representative miRNAs (miR-337-5p, miR-127-3p, miR-379-5p, miR-541-5p), a phenomenon observed not only in cardiac fibroblasts, but also in other organs such as skeletal muscle, liver and cardiac ventricles (Fig.7).

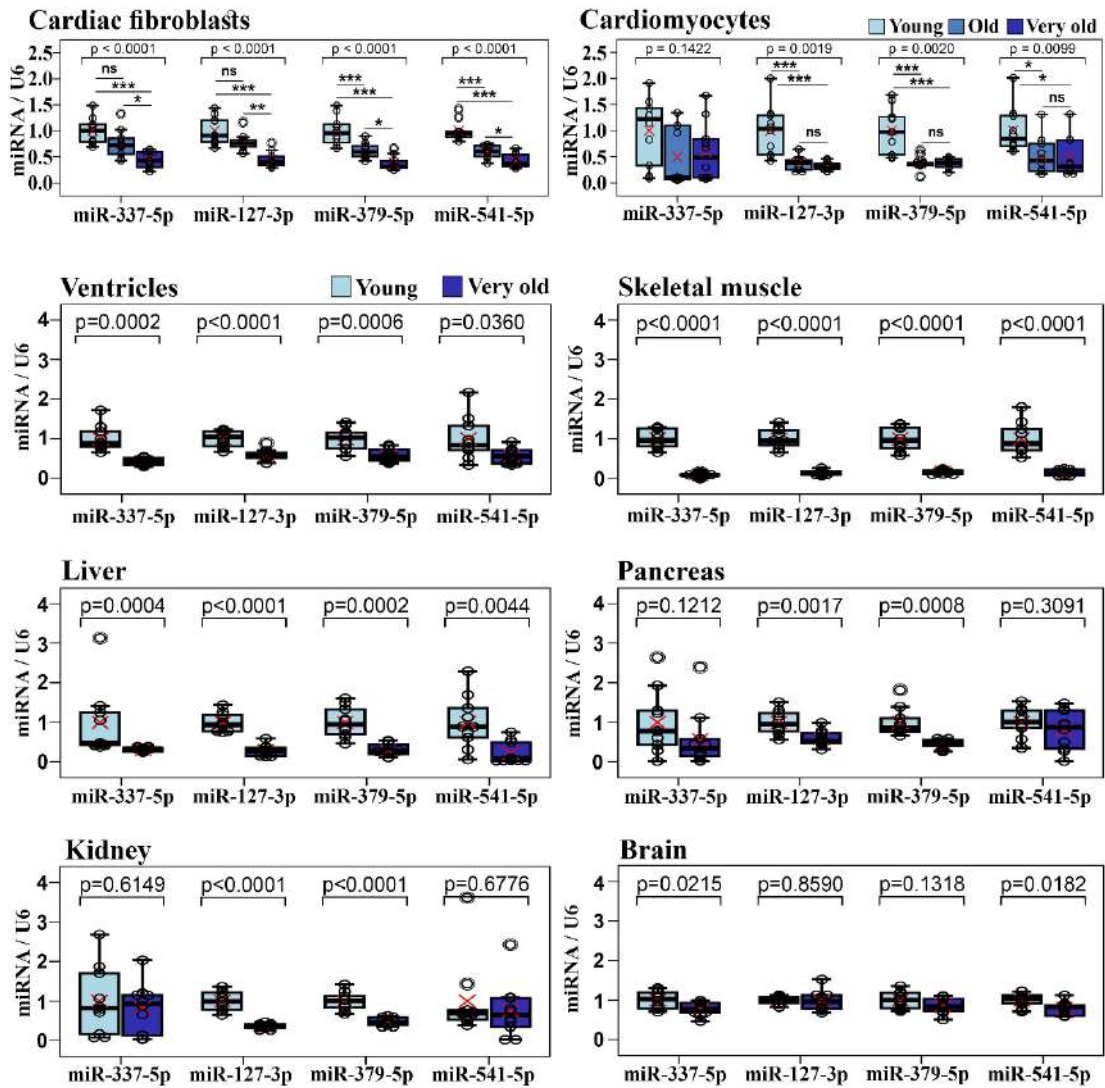
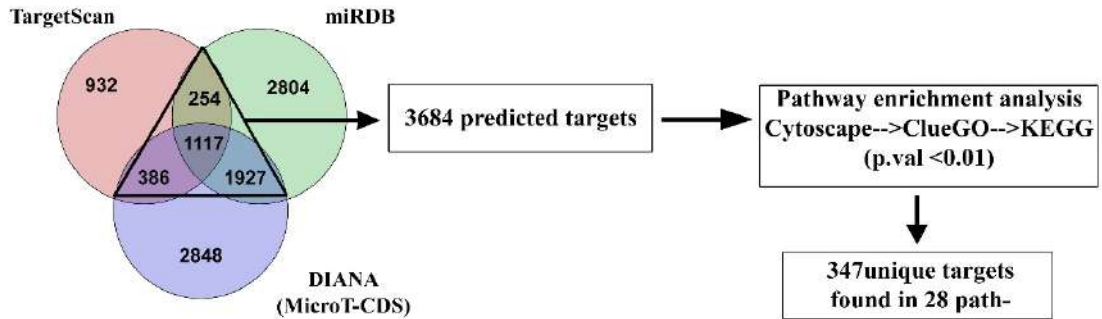


Figure 7. RT-qPCR Analysis of the four representative miRNAs from the Meg3-Mirg locus in cardiac cells and multiple organs collected from young and old mice. For each miARN, the expression level is shown relative to the young group; $n=8-10$ /group. Data are represented as mean $2^{-\Delta\Delta Cq}$ values, \pm SD.

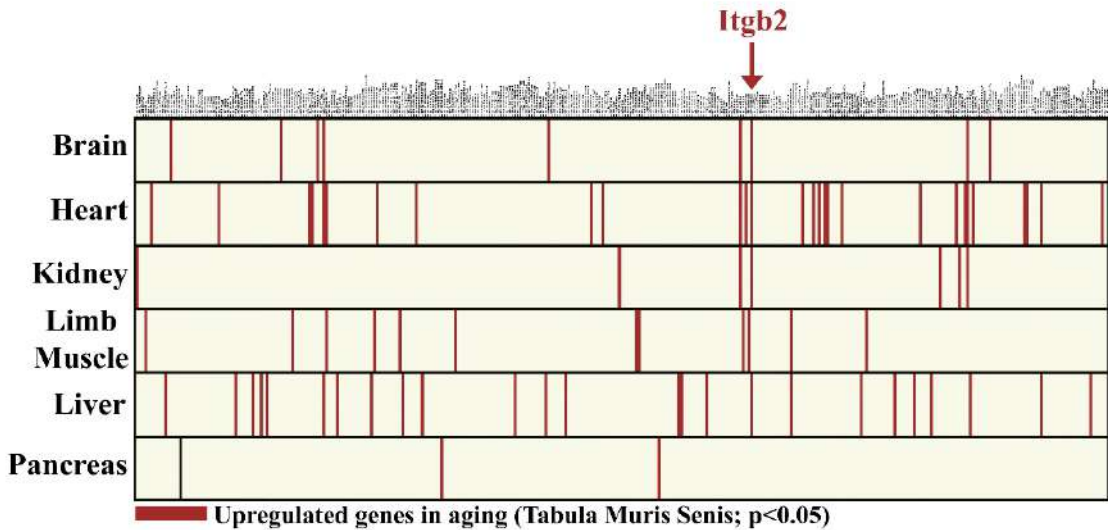
Subsequently to determine the biological role of the downregulation of miRNAs from the Meg3-Mirg locus, 27 representatives of this locus were included in an in silico analysis to identify their predicted target genes. Bioinformatic analysis revealed that the predicted targets of all miRNAs were significantly enriched in KEGG pathways related to longevity, inflammation and fibrosis, suggesting a central role of this locus in regulated age-associated processes (Fig. 8A). Furthermore, by intersecting the target genes of the 27 miRNAs with the genes unregulated during ageing from the Tabula Muris Senis data set, we identify and validated *Itgb2* (Fig. 8B-C) as a relevant target gene of Meg3-Mirg miRNAs, and found it

to be overexpressed in several aged organs (heart, brain, kidney pancreas and cardiac fibroblast).

A. Predicted targets of 27 downregulated miRNAs from Meg3-Mirg locus



B.



C.

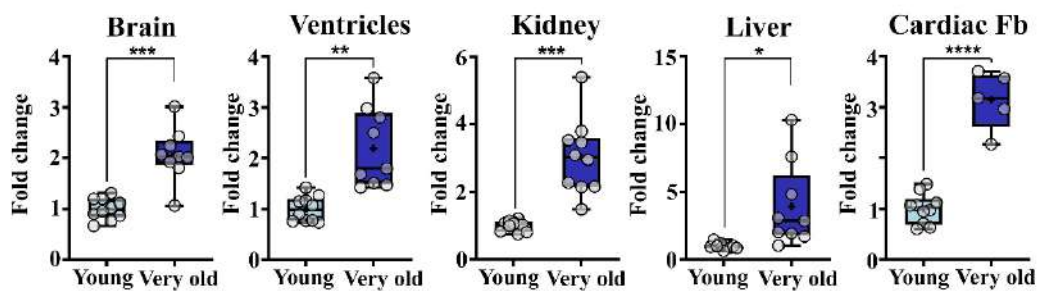


Figure 8. Gene upregulated during ageing that are targeted by the miRNAs from the Meg3-Mirg locus. (A.) Venn diagram illustrating the predicted targets of 27 miRNAs; (B.) Graphical representation of the intersection between the unique gene identified in KEGG pathways and the genes that are upregulated in the Tabula Muris Senis database. Upregulated genes are highlighted for each organ. *Itgb2* was identified as the only gene

upregulated in 4 out of 6 organs; (C.) The expression *Itgb2* gene in the brain, ventricles, kidney, liver and cultured cardiac fibroblasts.

Chapter 3 - INVESTIGATION OF MIRNAS WITH INCREASED EXPRESSION IN CARDIAC FIBROBLASTS DURING NATURAL AGEING. This chapter includes the study “*Activation of miR-10b in cardiac fibroblasts attenuates the adverse remodelling after myocardial infarction during aging by targeting Lpar2*”. In this study, we focused on identifying the roles of the upregulated miRNAs in cardiac fibroblasts, in the process of post-MI remodelling during ageing. Using in vivo functional studies, molecular biology techniques, biochemistry, cloning and cell culture experiments, we identified miR-10b-5p as being overexpressed in cardiac fibroblasts (cFbs) during ageing (Fig.9A). The increase was also confirmed in the aged heart and was attributed exclusively to the accumulation of miR-10b in cardiac fibroblasts, and not in cardiomyocytes, suggesting an important role for this miRNA in the fibrotic remodelling process associated with ageing (Fig.9B).

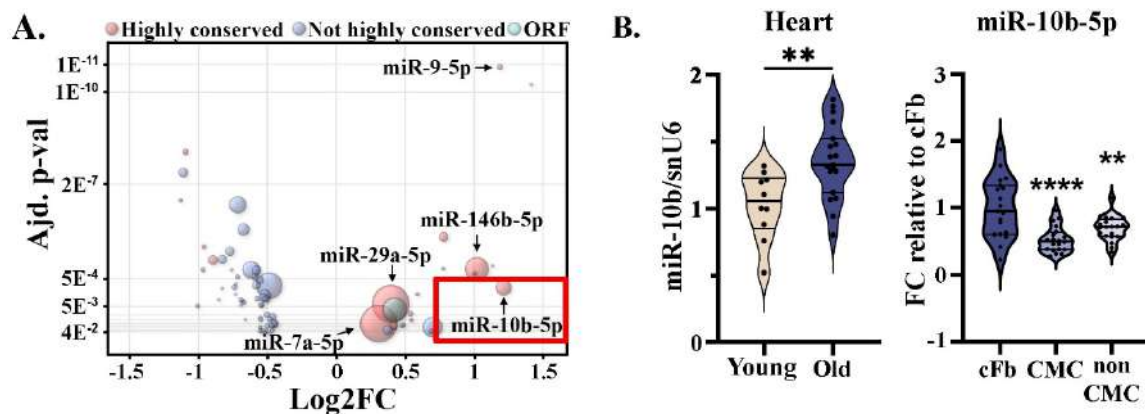


Figure9. Dysregulation of miR-10b expression during cardiac ageing. (A.) Volcano bubble plot showing the dysregulated miRNAs in cardiac fibroblasts during ageing. Bubble size represents the mean expression level (RPM-reeds per million), while the colour indicates the conservation status: red= highly conserved (across vertebrates); blue= conserved only in mammals; (B.) Relative expression of miR-10b in the heart and isolated cardiac cells from young and old mice. $N=10-20/\text{group}$. Data are presented as mean $2^{-\Delta\Delta Cq}$ values \pm SD.

Thus, we hypothesized that miR-10b expression might also be altered in post-MI cardiac fibrosis. Our data showed that miR-10b exhibited a biphasic regulation during myocardial infarction being reduced during the inflammatory phase (1-3 days post-MI) and increased during the reparative/maturation phase (7-14 days post- MI) (Fig.10A-B). We showed that inflammatory signals such as TNF-alpha inhibited its expressions, thereby facilitating

fibroblast activation and matrix deposition. In contrast, increased levels of miR-10b in the presence of inflammatory stimuli promoted fibroblast apoptosis (Fig.10C). Furthermore, we demonstrated that in vitro overexpression of miR-10b in cardiac fibroblasts exerts an anti-fibrotic role by inhibiting their proliferation and migration and by reducing ECM molecules, such as *Ctgf* (Fig.10D-E). Collectively, these miR-10b - mediated processes may contribute to stabilising the post-MI scar and preventing excessive ECM accumulation.

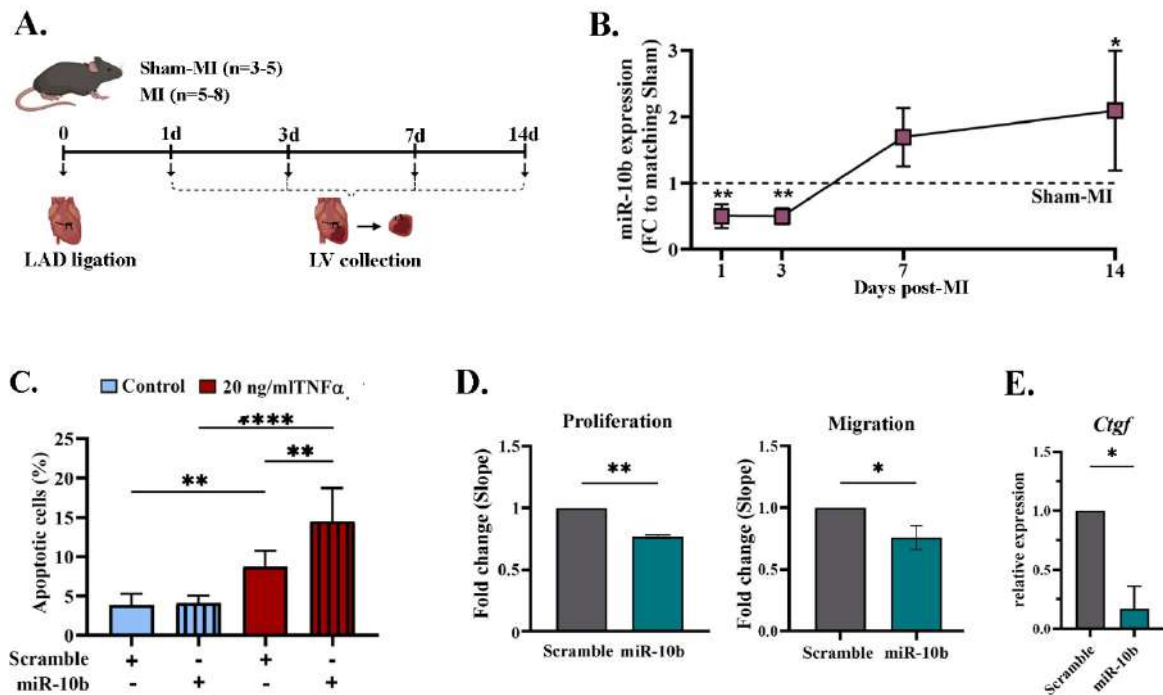


Figure 10. The expressions and role of miR-10b during post-MI reparative phases. (A.) Schematic representation of the experimental design. Myocardial infarction was induced in young C57Bl/6 mice (2-3 months) by ligation of the left anterior descending coronary artery (LAD). The infarcted region on the left ventricle was collected in liquid nitrogen at 1, 3, 7 and 14 days post-MI; (B.) miR-10b expression at different time intervals after MI. Data are calculated relative to the corresponding Sham group at each collection time point (mean \pm SD; n=3-9); (C.) The impact of miR-10b overexpression in 3T3 fibroblasts on cell apoptosis under inflammatory conditions; (D.) The effect of miR-10b on proliferation and migration of cardiac fibroblasts using the xCELLigence RTCA Biosensor system. Bar plots shows slope analysis describing the inclination, gradient and rate of change of the cell index curves measured over time. Three independent experiments were performed and slope results for each experiment were normalized to 1; (E.) Evaluation of *Ctgf* expression following miR-10b transfection in immortalized aged cardiac fibroblasts.

To validate this hypothesis, we assessed the contribution of miR-10b in post-MI ventricular remodelling. For this purpose, we investigated the underlying mechanisms through which this miRNA influences ischemia-induced ventricular remodelling during aging. The results showed that miR-10b was upregulated in cardiac fibroblasts isolated 7 days post-MI from the infarcted region (Fig.11A). Bioinformatic analysis and functional assays further identified Lpar2 as a direct target of miR-10b (Fig.11B). Accordingly, overexpression of miR-10b in aged cardiac fibroblasts reduced Lpar2 at both the transcript and protein level (Fig.11C), and luciferase reporter assays confirmed the direct miR-10b-Lpar2 interaction (Fig.11D). To further elucidate the molecular mechanisms by which the LPA/Lpar2-miR-10b axis regulates cardiac fibrosis, we explored the role of lysophosphatidic acid (LPA) in this process. LPA is a bioactive phospholipid with growth factor-like properties, known to stimulate cell proliferation, migration and survival. Moreover, LPA signalling, mediated through its receptors (Lpar1-6), plays a significant role in the development of fibrosis.

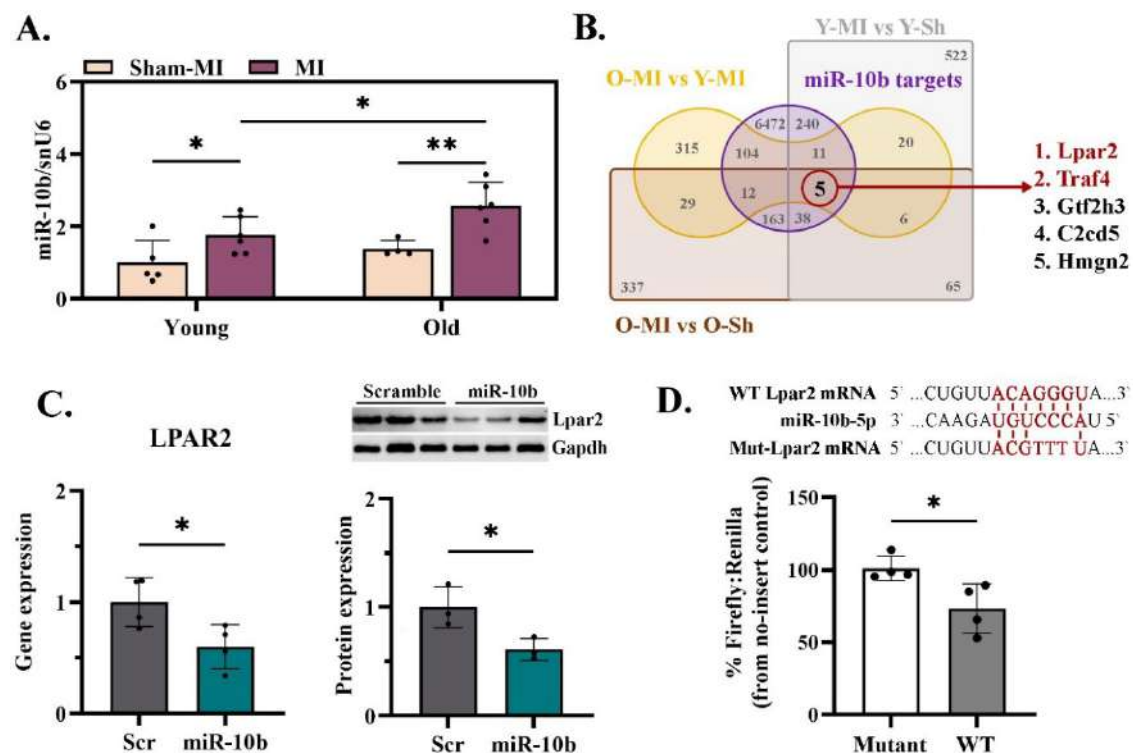


Figure 11. Identification and validation of Lpar2 as a target of miR-10b. (A.) RT-qPCR analysis of miR-10b expression in cardiac fibroblasts isolated from young and old mice 7 days post-MI; (B.) Bioinformatic analysis to identify target genes of miR-10b. The genes found downregulated in the experimental groups Y-MI vs Y-Sham, O-MI vs O-Sham and O-MI vs Y-MI were overlapped with the predicted miR-10b targets identified from four databases; (C.) Gene and protein expression of Lpar2 in old cardiac fibroblasts

overexpressing miR-10b; (D.) Dual-luciferase reporter assay demonstrating the direct interaction between *Lpar2* and miR-10b-5p. The pmirGLO reporter plasmid containing the seed sequence of the *Lpar2*-WT or *Lpar2*-Mut was co-transfected with miR-10b into HEK293 cells.

Functional essays showed that in the presence of a specific *Lpar2* agonist, miR-10b overexpression reduced both proliferation (Fig.12A) and the expression of Collagen I and III genes (Fig.12B) in aged cardiac fibroblasts, further supporting the role of this pathway in fibrosis.

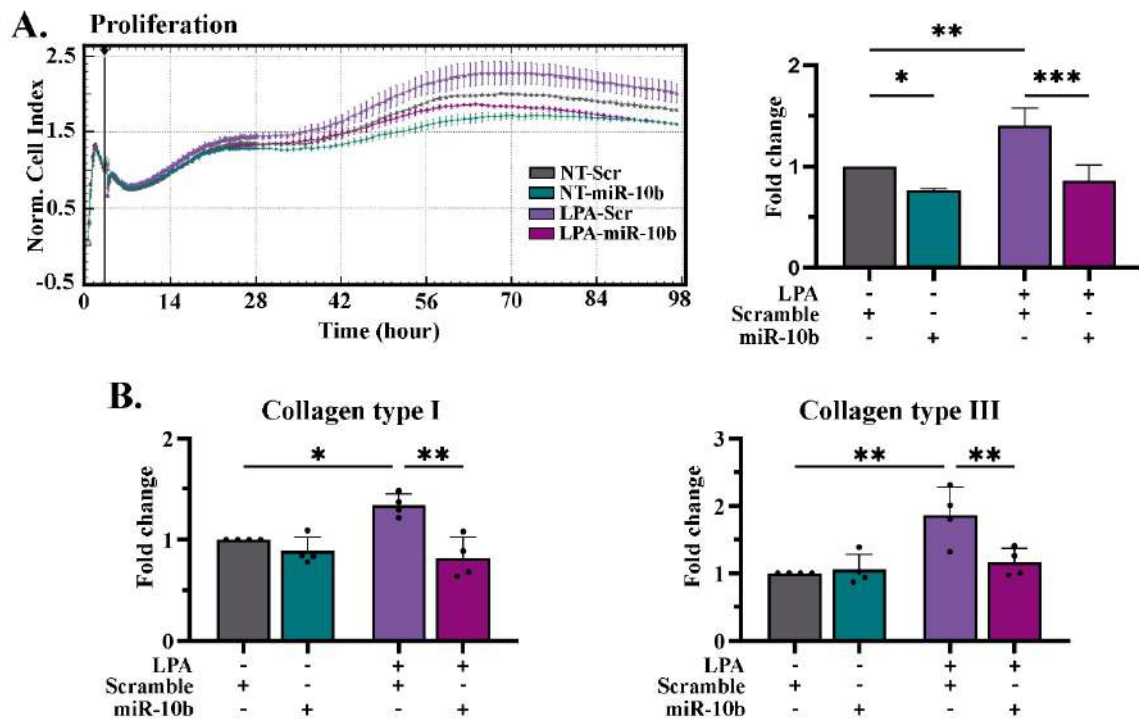


Figure 12. The role of the miR-10b/*Lpar2* axis in the fibrotic process. (A.) Cells were transfected with miR-10b or Scramble for 24 hours and subsequently exposed to the LPA2 agonist for 98 hours. The left plot shows the normalised cell index, represented as mean \pm SD of two or three individual wells from a representative experiment. The right graph quantifies the slope of proliferation curves from three independent experiments, describing the gradient and rate of change of the cell index curves over the 98 hours treatment period. Results are reported relative to the Scramble control for each experiment; (B.) Relative quantification of Collagen I and III genes in immortalised aged cardiac fibroblasts overexpressing miR-10b after 48 hours of treatment with the LPA2 agonist.

Finally, we demonstrated that *in vivo* inhibition of miR-10b using an antagomir (Fig.13A-B) exacerbated the adverse remodelling post-MI in aged mice, leading to significant structural changes in the left ventricle (LV). Specifically, an increase in LV area and diameter was observed, along with the reduction in the anterior wall thickness in both systole and diastole. In addition, both end-diastolic and end-systolic volumes were increased in the anti-10b group (Fig.13C). These results showed that miR-10b inhibition exacerbates the adverse cardiac remodelling after myocardial infarction, manifested primarily by ventricular dilation and define the miR-10b-Lpar2 axis as a critical regulatory mechanism of fibroblast proliferation and extracellular matrix deposition in ageing, with potential for therapeutic exploitation.

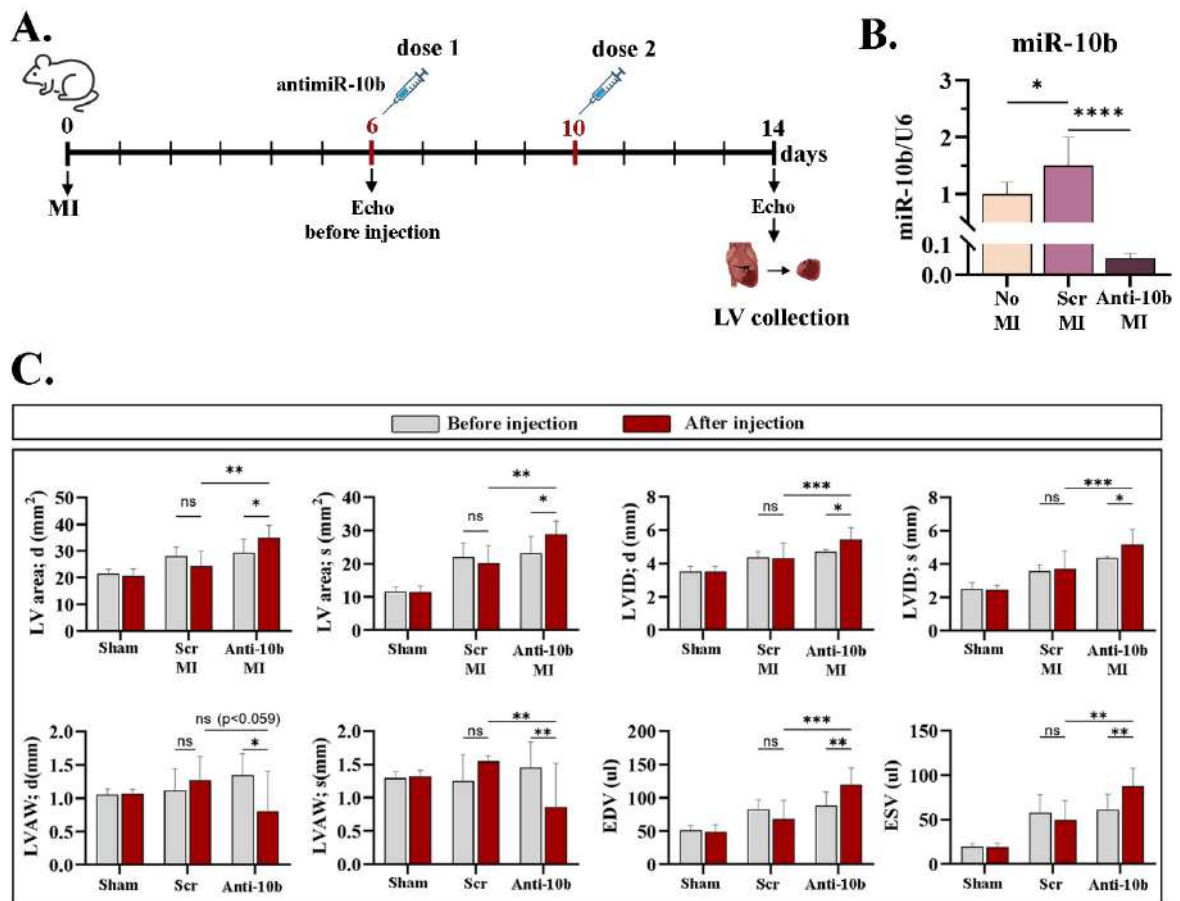


Figure 13. *In vivo* inhibition of miR-10b after MI. (A.) Pictogram illustrating the experimental design. Aged C57Bl/6 mice (17-18 months) underwent either Sham or MI induction surgery. A dose of 10 mg/kg Scramble (Scr) or AntagomirR-10b (Anti-10b) was administered to the MI group after 6- and 10-days post-procedure; (B.) The expression level of miR-10b in the left ventricle after myocardial infarction; (C.) Measurements of LV area, LV internal diameter (LVID), LV anterior wall thickness (LVAW) and end-systolic (ESV)

and end-diastolic (EDV) volumes over a cardiac cycle. Measurements were performed before the first dose of treatment and at the end point of the experiment (after injection).

Original contributions of the thesis

- Standardisation of the protocols for the isolation of adult murine, cardiac fibroblasts and cardiomyocytes for in vitro studies;
- We demonstrated that cardiac fibroblasts from aged heart has a frailty phenotype characterised by stress fibre reorganisation and metabolic changes with a shift towards glycolysis and loss of energetic flexibility under stress conditions;
- We identify and characterise the miRNAs expression profiles in cardiac fibroblasts isolated from young and old mice;
- We identify miR-29a as a globally upregulated miRNA during ageing, exerting a compensatory role with anti-fibrotic effects in this process;
- We demonstrated that miR-10b is increased in the aged male heart due to its overexpression in cardiac fibroblasts, and not in cardiomyocytes;
- We identify a temporal regulation of miR-10b, with its expression reduced during post-MI inflammatory phase and increased during the reparative and scar maturation phase;
- We demonstrated that the exposure of cardiac fibroblasts to inflammatory stimuli, such as TNF-alpha decreased its expression, while its overexpression under the same conditions promoted cellular apoptosis;
- We demonstrated that miR-10b has an anti-fibrotic role under basal conditions in cardiac fibroblasts by reducing proliferation, migration and *Ctgf* expression;
- We demonstrated that the activation of Lpar2 receptor using an LPA2 agonist is inhibited by miR-10b overexpression in cardiac fibroblasts, leading to reduced cell proliferation and decreased expression of profibrotic genes such as Collagen I and Collagen III, thereby validating the anti-fibrotic role of miR-10b;
- We demonstrated that in vivo inhibition of after MI exacerbates fibrotic remodelling during ageing.

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EFFECTIVENESS AND DISSEMINATION OF RESEARCH

Papers published in ISI journals- 2 as first author

1. **Rusu-Nastase EG**, Lupan AM, Marinescu CI, Neculachi CA, Preda MB, Burlacu A. *MiR-29a Increase in Aging May Function as a Compensatory Mechanism Against Cardiac Fibrosis Through SERPINH1 Downregulation*. *Frontiers in Cardiovascular Medicine*, 2022 doi: 10.3389/fcvm.2021.810241. **Journal impact factor (2022): 3.6 (Q2)**
2. Lupan AM*, **Rusu EG***, Preda MB, Marinescu CI, Ivan C, Burlacu A. *miRNAs generated from Meg3-Mirg locus are downregulated during aging*. *Aging (Albany NY)*, 2021, doi: 10.18632/aging.203208 (* equal contributions). **Journal impact factor (2021): 5.682 (Q1)**

Manuscript in preparation: **Rusu-Nastase EG**, Marinescu CI, Neculachi CA, Preda MB, Martelli F, Burlacu A. *MiR-10b activation in cardiac fibroblast attenuates the adverse cardiac remodelling after myocardial infarction during ageing by targeting Lpar2*.

Papers published in ISI journals – 5 as co-author

1. Laudy Cherry, Sinziana Popescu,&, Carmen Alexandra Neculachi, **Evelyn-Gabriela Nastase-Rusu**, Catalina Iolanda Marinescu-Colan, Bogdan Paul Cosman, Letitia Ciortan, Fabio Martelli, Maya Simionescu, Elena Butoi, Alexandrina Burlacu, Mihai Bogdan Preda. *Mesenchymal stromal cells modulate neutrophil phenotype via paracrine signals*. *Stem Cell Research & Therapy*, 2025. **Journal impact factor (2024): 7.3 (Q1)**.
2. Neculachi CA, **Nastase-Rusu EG**, Cherry L, Marinescu-Colan CI, Tastsoglou S, Cosman BP, Popa AM, Panciuc C, Zaccagnini G, Catrina SB, Simionescu M, Martelli F, Preda MB, Burlacu A. *miR-210 promotes the anti-inflammatory phenotype and M2 polarization in murine macrophages*. *Frontiers in Immunology*. 2025; doi: 10.3389/fimmu.2025.1633163. **Journal impact factor (2024): 5.9 (Q1)**.
3. Preda MB, **Nastase-Rusu EG**, Neculachi CA, Zhong X, Voellenkle C, Mazure NM, Balacescu O, Ivan C, Zheng XW, Gherghiceanu M, Lebrigand K, Simionescu M, Martelli F, Mari B, Catrina SB, Burlacu A, Ivan M. *miR-210 locus deletion disrupts cellular homeostasis: an integrated genetic study*. *Scientific Reports*. 2025;15(1):22659. doi: 10.1038/s41598-025-07572-8. **Journal impact factor**

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4. Marinescu-Colan CI, Nastase-Rusu EG, Neculachi CA, Martelli F, Cherry L, Preda MB, Burlacu A. *From cancer to heart fibrosis - GLIPR1 highlights a subset of myofibroblasts responsive to mesenchymal stem cell therapy after myocardial infarction*. *Biomedicine & Pharmacotherapy*, 2025; doi: 10.1016/j.biopha.2025.118087. **Journal impact factor (2024): 7.5 (Q1)**.
5. Marinescu CI, Preda MB, Neculachi CA, Rusu EG, Popescu S, Burlacu A. *Identification of a Hematopoietic Cell Population Emerging From Mouse Bone Marrow With Proliferative Potential In Vitro and Immunomodulatory Capacity*. *Frontiers in Immunology*, 2021, doi: 10.3389/fimmu.2021.698070. **Journal impact factor (2021): 7,561(Q1)**
6. Preda MB, Lupan AM, Neculachi CA, Leti LI, Fenyó IM, Popescu S, Rusu EG, Marinescu CI, Simionescu M, Burlacu A. *Evidence of mesenchymal stromal cell adaptation to local microenvironment following subcutaneous transplantation*. *Journal of Cellular and Molecular Medicine*, 2020, doi: 10.1111/jcmm.15717. **Journal impact factor (2020): 5.310 (Q2)**

Oral communications at international scientific conferences: 2 oral presentations

1. Rusu Evelyn-Gabriela. Oral presentation during the "39th Annual Scientific Session of the Romanian Society for Cell Biology". *Natural aging process is associated with increased miR-29a and downregulated SERPINH1 in multiple organs*, 21-23 October 2022, Cluj, Romania- **awarded with „Best Presentation Award” in the PhD Students Presentations session**.
2. Rusu Evelyn-Gabriela. Oral presentation during the "38th Annual Scientific Session of the Romanian Society for Cell Biology". *MiRNAs generated from Meg3-Mirg locus are downregulated during aging*, 4-6 November 2021, Bucharest, Romania.

Posters presented at international scientific conferences: 5 posters

1. Rusu E.G., Burlacu A. *Evaluation of the impact of alginate-based scaffolds on the mesenchymal stem cell properties in vitro*, presented at "The 10th National Congress with International Participation and 36th Annual Scientific Session of RSCB", 5-9 June 2018, Craiova, Romania.
2. Rusu E.G., Lupan A.M, Marinescu C.I, Preda B.M, Burlacu A. *Influence of aging on the quality and dynamics of mouse cardiac fibroblasts in culture* presented at the "European Research Area Network (ERA-Net) on Cardiovascular Diseases

(ERA-CVD) meeting”, 13-15 May **2019**, Riga, Latvia.

3. **Rusu E.G.**, Lupan A.M, Marinescu C.I, Preda B.M, Burlacu A. *Influence of aging on the quality and dynamics of mouse cardiac fibroblasts in culture* presented at the” International Cell Culture Under Flow Meeting 2020”, 18-19 February **2020**, Munich, Germany. **Poster presentation awarded with the „Young Scientist Award”**.
4. **Rusu E.G.**, Lupan A.M, Marinescu C.I, Preda B.M, Burlacu A. *MiRNAs generated from Meg3-Mirg locus are downregulated during aging. 2nd Olympiad in Cardiovascular Medicine* presented at the” International Symposium on Experimental and Clinical Cardiology”, 27-30 April **2022**, Heraklion, Crete
5. **Rusu E.G.**, Lupan A.M, Preda B.M, Marinescu C.I, Neculachi C.A, Burlacu A. *Natural aging process is associated with increased miR-29a and downregulated Serpinh1 in multiple organs* presented at the ”Final CardioRNA COST Action Meeting”, 22-24 April **2023**, Nicosia, Cyprus.

Specialisations and courses completed during the doctoral programme

1. “CardioRNA Bioinformatics Training School” organized by COST CA17129 “CardioRNA”, Nicosia, Cyprus, 20-22 February 2023.
2. ”10th International Course: Care and use of laboratory animals (Mice, Rats, Zebrafish)” Crete, Greece, 17 April-7 June 2024.

Awards

1. ” **Young Scientist Award**” for the poster: **Rusu E.G.**, Lupan A.M, Marinescu C.I, Preda B.M, Burlacu A. *Influence of aging on the quality and dynamics of mouse cardiac fibroblasts in culture*, presented during” International Cell Culture Under Flow Meeting 2020”, 18-19 February **2020**, **Munich, Germany**
2. ” **Best Presentation Award**” for the oral presentation: **Rusu Evelyn-Gabriela**. *Natural aging process is associated with increased miR-29a and downregulated SERPINH1 in multiple organs*, presented during ”39th Annual Scientific Session of the Romanian Society for Cell Biology”, 21-23 October **2022**, **Cluj, Romania**.

RESEARCH FUNDING AND COLLABORATIONS IN RESEARCH PROJECTS

1. **PhD fellowship:** Romanian Academy (SCOSAAR): 2018-2021 (contract nr 88/04.11.2018)
2. **PNRR-III-C9-2022-I8-186** - *"Targeting Cardiac Fibrosis in Heart Failure; Challenges and Potential Solutions Based on ncRNA Therapeutics (FibroThera)"*, project leader Dr. Fabio Martelli, Scientific Director, Laboratory of Molecular Cardiology, I.R.C.C.S. Policlinico San Donato
3. **PN-III-P4-ID-PCCF-2016-0172** - *"Targeting innate immune mechanisms to improve risk stratification and to identify future therapeutic options in myocardial infarction"* (INNATE-MI), project leader Acad. Maya Simionescu (collaborator in the project implementation team in 2022)
4. **PN-III-P4-ID-PCE-2020-1340** - *"Fundamental mechanisms of ventricular remodelling evaluated in cardiac fibroblast population in the aged myocardium with infarction (Inf-OLD)"*, project leader Dr. Alexandrina Burlacu
5. **PN-III-P1-1.1-TE-2019-1893** - *"MiR-210 genomic locus. and the overlap between hypoxia signalling and inflammatory networks"* (210-HINT), project leader Dr. Bogdan Preda
6. **COFUND-ERA-CVD-EXPERT-65/2017** - *"Exploring new pathways in age-related heart diseases"*, ERA-CVD Joint Transnational Projects 2017-2020; (1) Hannover Medical School; (2) Maastricht University; (3) Humanitas Research Hospital; (4) Centro Nacional de Investigaciones Cardiovasculares Carlos III; (5) University of Lorraine; (6) Institute of Cellular Biology and Pathology (PI: Dr. Alexandrina Burlacu)
7. **POC-A.1-A.1.1.4-E-2015** – *"Improving institutional competitiveness in the field of type 1 diabetes through the development of an innovative mesenchymal stromal cells-based immunotherapy concept (DIABETER)"*, project leader Dr. Nadir Askenasy (Frankel Laboratory for Experimental Bone Marrow Transplantation, Schneider Children's Medical Center of Israel). Internal scientific coordinator: Dr. Alexandrina Burlacu.