A Demonstration for Content Delivery on Wi-Fi Direct Enabled Devices

A computationally inferred regulatory heart aging model including post-transcriptional regulations

G. Politano*, F. Lograndᵗ, M. Brancaccioᵗ and S. Di Carlo*

*Control and Computer Engineering Department, Politecnico di Torino, Torino, Italy
ᵗDepartment of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy

***Abstract*—Cardiovascular diseases are one of the leading causes of death in most developed countries and aging is a dominant risk factor for their development. Among the different factors, miRNAs have been identified as relevant players in the development of cardiac pathologies and their ability to influence gene networks suggests them as potential therapeutic targets or diagnostic markers. This paper presents a computational study that applies data fusion techniques coupled with network analysis theory to identify a regulatory model able to represent the relationship between key genes and miRNAs involved in cardiac senescence processes. The model has been validated through an extensive literature analysis that was able to connect 94% of the identified genes and miRNAs with cardiac senescence related studies.**

***Index Terms*—Senescence, heart, regulation, pathway, microrna.**

I. Introduction

Aging affects several biological processes with a progressive impairing of key physiological functions, reduced response to stress and susceptibility to various diseases [1]. Cardiovascular diseases are one of the leading causes of death in most developed countries and aging is a dominant risk factor for their development [2]. Identification of regulatory mechanisms contributing to cardiac aging may help the identification of early cardiovascular pathophysiological changes.

Different studies proposed potential causes for cardiac aging. These include decrease in telomerase activity and shortening of the DNA of the telomeres [3], increased oxidative stress [4], loss of mitochondrial function [5] and impaired autophagy [6]. Moreover, epigenetic regulators such as microRNAs (miRNAs) have been studied in several cellular aging processes [7]. MiRNAs control the expression of genes involved in several key biological processes such as cell development, stem cell proliferation, division and differentiation, regulation of immunity, apoptosis, cell signaling and metabolism. Aberrant expression of miRNAs has been associated with several pathological processes [8]. MiRNAs substantially contribute to the development of cardiac pathologies and their ability to influence gene networks suggests them as potential therapeutic targets or diagnostic markers [9], [10].

In this paper we propose a computational study that applies data fusion techniques coupled with network analysis theory to identify a regulatory model able to represent the relationship between key genes and miRNAs involved in cardiac senescence processes. This model represents a very preliminary outcome that could help in the identification of molecular mechanisms and pathways involving miRNAs responsible for cardiac aging processes.

Contact: stefano.dicarlo@polito.it

II. Materials and methods

We developed a semi-automated pipeline able to identify genes with key regulatory role in an aggregated multi-pathway gene regulatory network (GRN). We started from a list of differentially expressed genes related to cardiac aging, which led to the identification of a subset of KEGG's pathways. By extending those pathways with their transcriptional and post-transcriptional regulators we were able to compute gene centrality measures across the pathways that eventually allowed us to produce a list of high centrality nodes. Such nodes resulted well interconnected, resembling a highly interconnected sub-network able to dispatch key regulatory signals previously associated with senescence [11]. Fig. 1 shows the overall data flow that takes advantage of two Cytoscape plugins: (i) ReNE [12], and, (ii) NetworkAnalyzer [13].

A. Data Selection

Two data sources were analyzed to identify the initial list of candidate genes:

- a list of differentially expressed genes. Genes related to cardiac aging in rat were extracted from GEO [14]. The selected experiment investigates effects of reduced adrenergic signaling in the aged heart [15][1]. Microarray data were filtered using the GEO Differential Expression filter. Furthermore, transcribed loci, pseudogenes, and expressed sequence tags were removed from the obtained list.
- a manually curated selection of pathways from KEGG [16]. The whole list of KEGG pathways was ranked according to the number of the previously identified genes they contain. The top 15 ranked pathways were analyzed performing a manual selection of interesting pathways.

B. Data Aggregation

The list of differentially expressed genes, and the selected KEGG pathways were individually processed resorting to the ReNE Cytoscape plugin [12]. The following operations were performed using ReNE on each pathway:

- *Naming normalization*: since different databases use different ways to identify genetic entities all symbols were converted according to their NCBI [17] and Uniprot [18] unique identifiers, thus allowing to navigate across public omic repositories without ambiguities.

[1]Data accessible at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE421, comparison of young (3-4 month) and aged (20-22 month) male Fischer-344 heart ventricle

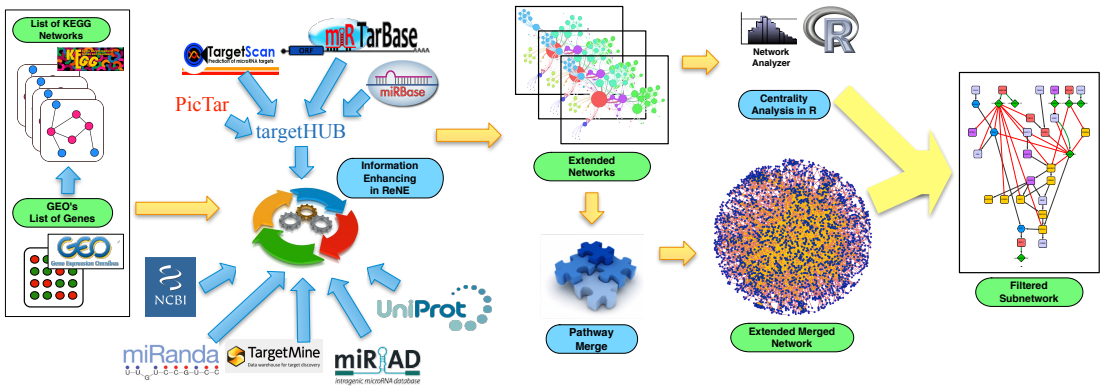

Figure 1. Green balloons identify Input/Intermediate/Output data; Cyan balloons identify processing activities. Pathways from KEGG and a list of differentially expressed genes from GEO were processed in ReNE, producing a set of extended networks. The extended networks were analyzed with NetworkAnalyzer and R scripts to identify nodes with high centrality score. The extended networks were merged obtaining an extended merged network modeling the whole set of regulations. Genes with high centrality (identified in the R processing step) were used to filter the extended merged network producing a filtered subnetwork containing the most central nodes possibly involved in cardiac aging.

- *Transcriptional enhancing*: transcription factors (TFs) of each gene were systematically retrieved from TargetMine [19] and integrated in the analyzed networks creating new TF nodes with outgoing edges directed to their target genes.
- *Post-transcriptional enhancing*: intragenic miRNAs hosted by the network genes were identified by ReNE from the miRIAD database [20] and inserted in the network. Intragenic miRNAs selected in this way are known to be co-expressed with their host genes [21]. miRNAs are commonly not reported in publicly available regulatory pathways. Nonetheless they act as regulatory hubs that lead to complex post-transcriptional regulatory motifs able to fine tuning genetic regulatory signaling cascades. For each identified miRNA the list of its targets was computed by ReNE by searching multiple miRNA target databases using the TargetHUB services [22], thus connecting miRNAs with their targets in the pathways.

C. Data Processing

Network topology parameters were computed for each processed network using the Cytoscape NetworkAnalyzer plugin [13]. Among the large amount of scores produced by NetworkAnalyzer, we selected the centrality measures since they have been demonstrated particularly useful to identify key players in biological processes [23], e.g., highly connected vertices in protein interaction networks are often functionally important and their deletion is often related to lethality [24]. NetworkAnalyzer provides two centrality measures: (i) closeness centrality, and (ii) betweenness centrality. The closeness centrality uses information about the length of the shortest paths within a network. However, since this distance is only defined for pairwise strongly connected vertices, it can only be used with strongly connected networks. On the other hand, the betweenness centrality quantifies the ability of a vertex to monitor the communication between other vertices. Every vertex that is part of a shortest path between two vertices can monitor the communication between them. Counting how many communications a vertex can monitor provides an intuitive definition of centrality: a vertex is central if it can monitor several communications between other vertices. [23]. Shortest-path betweenness centrality was applied to mammalian transcriptional regulatory networks and it was noted that betweenness appears to be an interesting topological characteristic in regard to the biological significance of distinct elements [25]. Betweenness centrality was therefore exploited to identify genes whose role is central enough to possibly induce misbehavior when ectopically expressed under aging conditions.

NetworkAnalizer results were processed with a custom R script that integrated the topological scores of every pathway by sorting and intersecting the nodes with the higher betweenness centrality. This produced a list of high centrality nodes. Such nodes are promising regulators with a key role in signaling cascades related to aging.

D. Finalization

High centrality nodes identified in the previous step were finally mapped to a global network. The ReNE Cytoscape plugin was used to merge all the analyzed pathways and the initial list of differentially expressed genes. The merging process produced a large network that was filtered in order to retain only the high centrality nodes thus reducing its complexity. This filtered subnetwork is a good candidate to highlight the most important inter-pathways regulatory entities, defined accordingly to their high centrality.

III. Results and Discussion

A set of 191 differentially expressed genes out of 8,799 available microarray data were identified using the GEO Differential Expression filter on the GSE421 Dataset. The list was further reduced removing non-genes references (see Section II-A) obtaining a list 177 candidate differentially expressed genes in heart aging. By performing naming normalization using ReNE the list of 177 genes was further reduced to

157 human homologs with a valid NCBI ID[2]. KEGG pathways were ranked according to these 157 genes (see Section II-A) and the 15 top ranked pathways were manually analyzed selecting the following 7 relevant pathways:

- *MAPK* (hsa04010): MAP kinases are involved in many different cellular functions like apoptosis, proliferation, survival, growth arrest, differentiation, motility, metabolism and senescence. Alterations in the MAPK signaling pathway have been reported in several tissues during aging [26].
- *Rap1* (hsa04015): promotes integrin and cadherin signaling and is activated by shear stress to regulate NO production in endothelial cells. *Rap1* deficiency in murine endothelium leads to endothelial dysfunction and hypertension, common diseases in the elderly [27].
- *Neuroactive ligand-receptor interaction* (hsa04080): cardiovascular diseases and aging are both associated with changes in the neurohumoral system, mainly adrenergic and renin-angiotensin systems. In particular, it has been reported a decrease in catecholamine-responsiveness in the elderly [28] and its regulatory effect in pathways disturbed in heart failure [29].
- *cAMP* (hsa04024): is a secondary messenger generated by adenylate cyclase, downstream to β-adrenergic signaling. Genetic inhibition of *cAMP* signaling in the mouse myocardium protects against heart failure, attenuates heart aging and prolongs lifespan (interestingly, in the heart of these mice, the *Raf1/MEK/ERK* and the *AKT* pathways are activated) [30].
- *PI3K-Akt* (hsa04151): controls a multitude of biological processes in myocardial cells, e.g., cardiomyocyte hypertrophy, survival, energy production, contractility, and response to stress. Some evidences indicate that attenuation of the *PI3K/AKT* pathway contributes to age related changes in myocardium [31].
- *Alzheimer's disease and Huntington's disease* (hsa05010, hsa05016): protein misfolding plays a crucial role in neurodegenerative diseases. Increasing evidences indicate that, in the myocardium, mechanical and oxidative stress together with other pathological conditions lead to protein misfolding and that misfolded proteins play a relevant role in the onset and progression of cardiomyopathies [32]. Recently, extensive links have been identified between Alzheimer's disease and cardiovascular diseases in large-scale genome-wide association studies [33].

The 7 selected pathways and the list of 157 genes were processed using ReNE as described in Section II-B. As a result, 3 new TF nodes and 7 miRNA nodes were globally inserted. Moreover, as a result of this process, the list of 157 differentially expressed genes was connected and transformed into a new pathway thus obtaining a list of 8 processed pathways qualitatively depicted in Fig. III-A. The 8 processed networks were analyzed using the Cytoscape NetworkAnalyzer as described in Section II-C to compute betweenness centrality of each node. Results from all pathways were merged to sort genes based on their betweenness centrality and the top 40 nodes were selected as high centrality nodes, i.e., nodes with high likelihood of having a key role in signaling cascades related to aging. Malfunctions in these nodes will easily lead to widespread functional misbehavior of the entire regulatory network.

The 8 pathways were then merged obtaining a very large network qualitatively reported in Fig. III-B. The network complexity was reduced by collapsing protein nodes into their related coding genes (protein information are only required by ReNE for intermediate processing) obtaining a network composed of 909 nodes (i.e., genes, TFs, and, miRNAs) and 6,475 edges. By filtering this complex network with the set of identified high centrality nodes we were able to extract a sub-network containing 35 interconnected nodes qualitatively depicted in Fig. III-C. A detailed view of the network depicting the 35 identified regulators and their interactions is reported in Fig. 3. This network represents a candidate inter-pathway regulatory model of senescence related signal cascades.

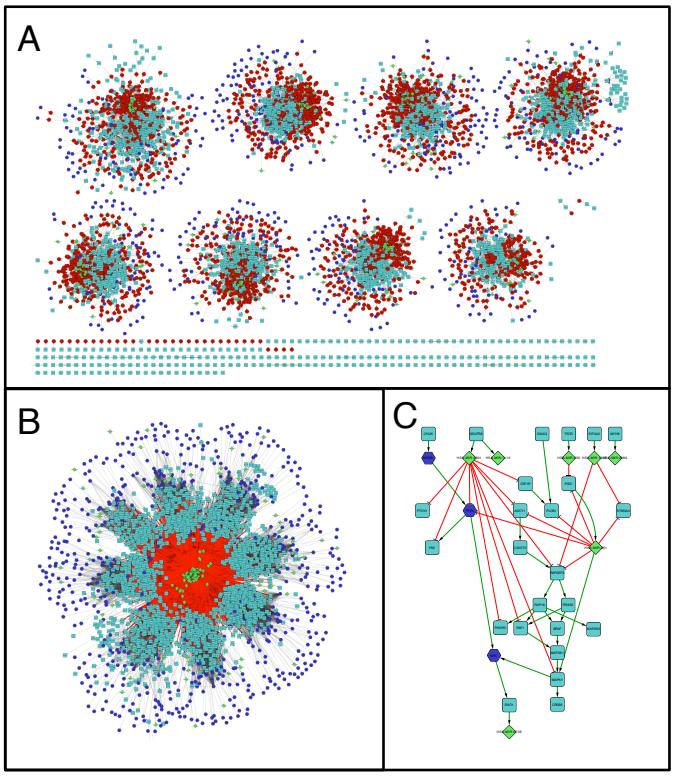

Figure 2. Conceptual processing steps. A – Seven KEGG pathways (hsa0401, hsa04015, hsa04024, hsa04080, hsa04151, hsa05010 and hsa05016), and a list of 157 differentially expressed genes (extracted from GEO) were processed using ReNE to add transcriptional and post-transcriptional regulators (green nodes: miRNAs, dark blue nodes: TFs, red nodes: proteins). B – the processed pathways were merged resulting in a large merged network. C – after centrality analysis genes with highest betweenness centrality were extracted along with their regulatory links, resulting in a small filtered network.

Literature validation confirmed that the nodes of the filtered subnetwork have been reported in different studies on heart aging response. The role of the identified genes, miRNAs and TFs was mapped, for the sake of clarity, against six main functional classes: 1) inflammatory response, 2) senescence regulation, 3) beta-adrenergic signaling, 4) *MAP2K* and *ERK1/2* signaling pathways, 5) miRNAs, and 6) TFs. Five

[2]The following entities have been discarded: Gstm7, LOC687048, LOC100365443, LOC100363469, LOC688869, Reg3b, LOC100364956, LOC100360403, Ly6al, LOC290595, Olr1642, RT1-M4, LOC100362894, Kng111

out of the seven identified miRNAs and all three identified TFs were reported as involved in senescence or inflammatory processes. Their role is of particular interest because they can finely tune the network behavior shading new light into the complex regulatory mechanisms underlying senescence. Overall, 32 out of the 35 network nodes (94%) have been previously reported in studies on aging related processes. The two missing nodes, i.e., miR-4691and miR-7113, represent two interesting candidates for future investigations. In particular, miR-4691 with his role of regulatory hub with 9 regulated nodes is a particularly interesting node to be investigated in laboratory.

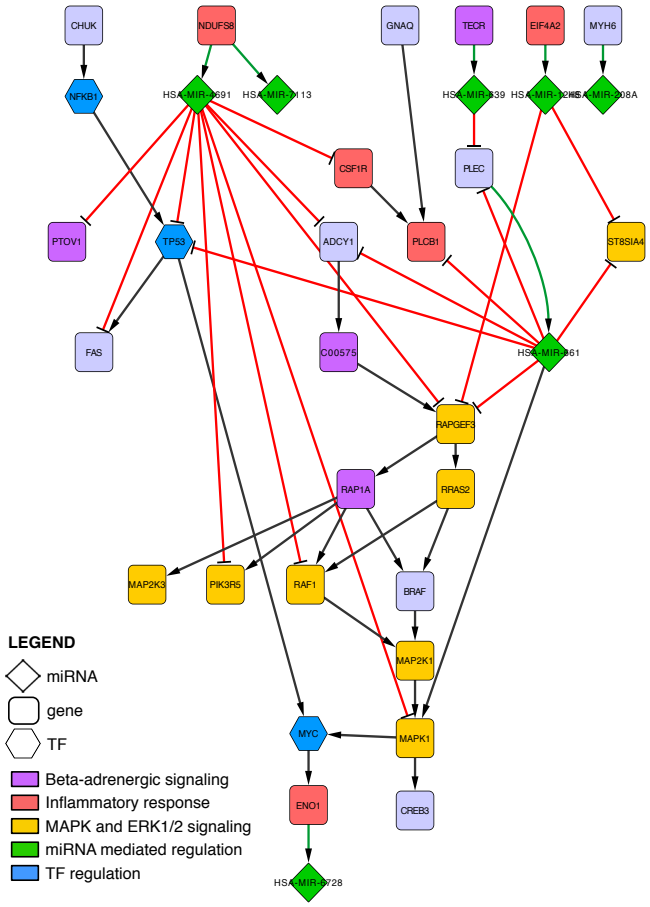

Figure 3. The resulting heart aging model. Nodes are colored to highlight their different roles: in purple Beta-Adrenergic signaling related nodes, inflammatory response in red, MAPK-Erk1/2 in yellow, senescence regulation in pale violet, microRNAs in green, and, transcription factors in blue. Edges are colored according to the following criteria: red for miRNA post-transcriptional repression, green for miRNAs co-transcription with their host gene, and black for regulations inherited from KEGG's pathways.

A. Inflammatory Response related genes

NDUFS8: mitochondria are necessary for the pro-inflammatory and pro-oxidant features of senescent phenotype. Their biogenesis stabilizes senescence via a crosstalk with the *ATM*, *Akt*, and *mTOR* signaling pathways. Since the increased mitochondrial mass is invariably reduced by rapamycin the *mTOR* pathway has a central role in mitochondrial regulation. *mTOR* inhibition results in a down expression of *mTOR* complex 1 (*mTORC1*), which integrates stress signals into the regulation of protein and lipid synthesis and autophagy, all of which are involved in the complex pathways mediating mitochondrial homeostasis. In this context, *NDUFS8* is a *mTORC1* downstream regulated gene and its presence may be an hallmark of mitochondrial regulation [34].

EIF4A2: is a member of the helicase family. Helicases are known as guardians of the genome given their role in various DNA metabolic pathways that include DNA recombination, replication, repair, transcription and telomere maintenance. *EIF4A2* seems related to aging by influencing protein translation and mitochondrial translocation efficiency [35].

ENO1: studies reported that *ENO1* is an auto-antigen that has been recognized as a common marker of systemic autoimmune diseases and inflammatory, degenerative and other pathological disorders. It is induced by cellular stress and promote cell growth, glycolysis, migration, and invasion [36].

CSF1R: the colony-stimulating-factor receptor-1 (*CSF1R*) is a type III receptor tyrosine kinase primarily reported as responsible for the proliferation, differentiation and survival of the monocyte–macrophage cell lineage, as well as their recruitment. Two ligands (*CSF1* and *IL34*) function through non-competitive binding to *CSF1R* by activating both phosphatidylinositol 3-kinase *PI3K*-dependent and *RAS*-activated protein kinase-dependent pathways. Senescent cells also revealed that *AKT* inhibition in *CSFR*-expressing cells is accompanied by the ectopic regulation of *CDKN1A*, *SOX2*, *OCT4* and *c-MYC* proteins. This establishes an interesting link between the expression of *CSF1R*, *AKT* activation and physical crosstalk between the *PI3K*, *AKT*, *STAT3*, and *NFKB* pathways [37].

PLCB1: acts as catalyst for the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. This reaction plays an important role in the intracellular transduction of many extracellular signals. *PLCB1* has been identified as a pro-inflammatory regulator involved in several cardiovascular diseases as acute, inflammatory, and self-limited vasculitis, Kawasaki Syndrome, endothelial cell inflammation with varying degrees of pro-inflammatory cytokine expression, and coronary artery aneurysm. [38]

B. Senescence Regulation

PLEC: laminar shear stress (LSS) caused by blood flow is known to regulate endothelial function and to contribute to vascular health. However endothelial cell senescence seems to increase the incidence of cardio vascular disorders. *PLEC1* is an actin binding protein induced in endothelial cells exposed to LSS. Studies demonstrated that several actin binding proteins including *PLEC1* may play a critical regulatory role in the endothelial wound healing under LSS conditions [39].

BRAF: constitutive activation of *BRAF* may drive the proliferation and maturation/senescence of melanoblasts. In mature human skin, descendant melanoblasts reside in the epidermis but they colonize all the tissues of the body, such as the valves of the heart and the meninges [40].

GNAQ: its mutations have been associated with defects in platelet activation and aggregation, and port-wine stains. It is involved in estradiol-responsive pathway and produces age-related differences in functional pathways related to oxidative phosphorylation, synaptic plasticity, and estrogen responsive

signaling cascades. *GNAQ* has also been recently reported as possibly responsible (with *NRAS*/*BRAF*-mutations) for transcriptional modifications in uveal melanoma and blue nevi that lead to melanoblast proliferation and melanocyte differentiation [40].

CREB3: is a regulator known to physically associate with the endomembrane system. It has been found ectopically expressed in abnormal lysosomes with impaired function. Abnormalities of lysosomal functions are characteristic of cellular senescence, organismal ageing, atherosclerosis, Alzheimer's and other diseases. Ectopic secretion of lysosomal proteases can, in fact, degradate extracellular matrix, contributing to atherosclerosis, arthritis, aneurysms formation, and other diseases [41].

MYH6: myosin-6 has been demonstrated ectopically regulated in senescent rats against young rats [42].

FAS: is a *TNF* receptor, shown to activate *NFKB*, *MAPK3/ERK1*, and *MAPK8/JNK* pathways. It is involved in transducing the proliferating signals in normal diploid fibroblast and T-cells. *FAS* appears impaired in senescence-associated secretory phenotype (SASP) proteases, i.e., cleave *FAS* ligand and other cell surface proteins required for effective immune function. The macrophage responses result declining with aging, potentially contributing to senescent cell accumulation in old age, which eventually results in a reduced immune function [43].

ADCY1: encodes a member of the of adenylate cyclase gene family. *ADCY1* has been identified as functionally responsible for enhanced forskolin-stimulated association with *ERK* that eventually contributes to *cAMP* elevation in vascular smooth muscle cells. *cAMP* over expression in the plasma membrane intensely strengthen the endothelial barrier function, resulting in cardiac rejuvenation, and suggests possibly regenerative potential in aged tissues. *ADCY1* ectopic activity is also proven sufficient to overwhelm the barrier protective effects of plasma membrane activity to promote endothelial gap formation [44].

CHUK: encodes a member of the serine/threonine protein kinase family. The encoded protein is an inhibitor of the transcription factor *NFKB* complex. In aging, reduced inhibition of NFKB is associated with the pro-inflammatory phenotype in vascular endothelium [45].

C. Beta-Adrenergic signaling

Chronic activation of beta-adrenergic signaling is deleterious to cardiac function resulting in increased heart rate, contractility, peripheral vasoconstriction and wall stress. Stimulation of beta-adrenergic receptors decreases anti-apoptotic and anti-oxidative stress signaling contributing to senescence and functional deterioration in a cascading effect with downstream induction of *cAMP* and *PKA* that act as inhibitors of the cardioprotective *Raf/MEK/Erk* pathways. In aging human heart, the functional responsiveness of the human cardiac beta-adrenoceptor system decreases along with all the effects mediated by *cAMP*.

PTOV1: androgens have been considered to reduce the incidence of ischemic myocardial disease in men, but they have also been reported to exert atherogenic effects on the human cardiovascular system through promoting plaque formation and enhancing monocyte adhesion to endothelial cells. In this context, *PTOV1* is considered to be one of the testosterone-induced genes involved in androgen-mediated stimulation of *VSMC* proliferation. *PTOV1* may thus play an important role in androgen-related atherogenesis in the male human aorta [46].

TECR: is a poorly characterized testosterone reduction enzyme member of steroid 5-alpha reductase family, which also contains *SRD5A2L2*, *SRD5A1*, *SRD5A2*, *SRD5A3*. *SRD5A1* and *SRD5A2* have been already identified as encoders for proteins targeted in treatments against prostate cancer and male pattern hair loss (male aging related diseases). However the role of *TECR* is still not clear. It has been shown to be involved in the elongation of very long chain fatty acids and reduction of a non-steroid lipid. *NbECR*, a *TECR* homolog in *Nicotiana Benthamiana*, has been identified as responsible for disorganized membrane structures and epidermal cell ablation. Those assumptions make *TECR* a candidate as processor for adrenergic and hormonal signals, capable to possibly lead to vascular membranes impairment [47], [48].

C00575 (cAMP): cAMP is a second messenger used for intracellular signal transduction such as transferring into cells the effects of hormones like glucagon and adrenaline, which cannot pass through the plasma membrane. *cAMP* also regulates the function of ion channels and a few other cyclic nucleotide-binding proteins such as *RAPGEF3*. Exchange proteins activated by *cAMP* (*RAPGEFx*), when *cAMP* binds, expose the activated *GEF* domain, allowing them to activate small *Ras*-like *GTPase*, such as *RAP1* [49].

RAP1: a *GTPase* superfamily member highly involved in cell motility and junction formation. It acts as a substrate for protein kinase A and interacts with *NADPH* oxidase (a group of multimeric enzymes whose activity results in the production of O2). In non-phagocytic cells, homologues of the *NADPH* oxidase were found in vascular endothelial cells and smooth muscle cells or transformed cells such as melanoma. Activation of *RAP1* strengthens cell junction integrity and reduces cell migration by affecting actin cytoskeleton remodeling [50]. *RAP1* also acts as a protective protein for telomeres by relocalizing to the upstream promoter regions of hundreds of new target genes when critical telomere shortening happens. *RAP1* role in delaying senescence and concurring in DNA damage responses makes it (and its surrounding regulators) a good candidate for actively managing senescence [51].

D. MAP2K and ERK1/2 signaling pathways

Activation of β-adrenergic receptors results in the production of cyclic *AMP* which in turn phosphorylates many transcriptional factors via the *MAPK* pathway and thus, stimulates protein synthesis and cell growth. In the filtered network, we identified a central cluster of genes that recurs in several high relevance pathways: *Ras*, *Raf*, *MAP2K*, and, *ERK1/2* signaling pathways. Such pathways are also highly interconnected to each other showing a concurrent role in crucial cell-survival regulations. At the cellular level, the *ERK1/2* pathway regulates cell cycle progression, proliferation, cytokinesis, transcription, differentiation, senescence, cell death, migration,

GAP junctions formation, actin and microtubule networks, and cell adhesion. *MAPK* pathway plays a role in many biological functions like cell cycle regulation, apoptosis, cell survival, senescence, differentiation, and cell growth and migration.

RRAS2: encodes a small *GTPase* involved in diverse processes including angiogenesis, vascular homeostasis and regeneration, and cell adhesion. *RAS* proteins interact with multiple effectors, including *RAF* kinases, *PI-3* kinases, and activate multiple downstream signaling cascades. Of particular interest the RAS/mitogen-activated protein kinase *RAS/MAPK* signaling pathway, which plays a central role in cellular proliferation and differentiation. *RRAS2* also plays a key role in Noonan syndrome, an autosomal-dominant disorder characterized by congenital heart defects. [52]

ST8SIA4: is predominantly expressed in immune cells. It plays an important role in substrate recognition that modulates cell adhesion and signaling. Furthermore, *ST8SIA4* mediates the activity of *PI3K/Akt* signal pathway in a competitive way. Inhibition of *PI3K/Akt* pathway is also able to attenuate the effects caused by the overexpression of *ST8SIA4* on *MDR* [53].

RAPGEF3: is necessary for antioxidant and antiapoptotic effects of exendin, which has an essential role in cardiomyocytes and is involved in the development of heart failure. *RAPGEF3* also drives the inhibitions of oxidative stress and apoptosis in cardiomyocytes thanks to *GLP-1R* which helps in cardioprotection, and also inactivates myosin light chain kinase [54]. Elevated levels of cAMP activate *RAPGEF3* which, in turn, leads to *GTP* binding to *Rap1*. This causes changes to the cortical cytoskeleton and organization of vascular endothelial cadherin in the endothelial junctions, leading to reduced endothelial permeability. Ectopic expression of *RAPGEF3* may thus result in cell aging effect [55].

RAF1: its activation engages the phosphorylation cascade starting from *RAF1* (*MKKK*) to *MAP2K*, and then to the *MAPK* family. *RAF1* was found to be an activator of *MAP2K* in mammalian cells and to form a stable complex with *MAP2K*. Prototypically, growth factors activate *RAS* which recruits and activates *RAF* at the plasma membrane. Once activated, *RAF* phosphorylates and activates *MAP2K*, which in turn activates *ERK1/2*. Eventually activated *ERK1/2* can phosphorylate downstream proteins in the cytoplasm or nucleus, including many transcription factors. Cardiac specific deletion of *RAF1* leads to heart failure without hypertrophy in the absence of external stress, and also increases apoptosis [56].

PIK3R5: activates the *Ras-Raf-MAP2K-ERK* signalling cascade. *PI3K* also represents a downstream target of active *RAS*. Suppression of *PI3K* prevents lipofuscin accumulation in aged heart tissue. Lipofuscin is thought to be generated from damaged proteins and post mitotic cells (such as cardiac myocytes) accumulate a large amount of lipofuscin in old age. Inhibition of *PI3K*, in fact, prevents the expression of cellular senescence markers (by decreasing the accumulation of lipofuscin in heart tissue) and most of the age-related changes of gene expression [57], [58].

MAPK1/MAP2K3/MAP2K1: the MAPK cascade is composed of *RAS*, *RAF1*, *MAP2K*, *MAPK1* and *RSK*. It spans from the plasma membrane to the nucleus and trasduces the mitogenic signals downstream from the tyrosine kinase membrane receptor. The first subgroup of *MAPK* (*ERK1/2*) plays an important role in the signaling pathway responsible for the *G0* to *G1* transition in the cell cycle. The MAPK1 pathway is coupled by a GTP-binding protein (Gαq-protein isoform in the heart) that activates the *ERK1/2* pathway. The activation of the *ERK1/2* pathway in heart is lead by a GTP-binding protein (Gαq-protein isoform) that produces diacylglycerol (*DAG*) and inositol-3,4,5-triphosphate (*InsP3*). *DAG* then activates the *MAPK1* signaling cascade, while *InsP3* activates the *MAPK1* pathway via both *RAS*-dependent and -independent mechanisms. The activity of *MAPK1* is stimulated by multiple extracellular stimuli and oncogenes. Its activation regulates several cellular protein kinases, and catalyzes the phosphorylation of some nuclear transcription factors [58]. Finally, mutations in *HRAS*, *KRAS*, *BRAF*, and *MAP2K* have also been discovered to be involved in other genetic disorders with cardiac developmental defects, such as LEOPARD syndrome, cardio-facio-cutaneous syndrome, and Costello syndrome [56].

E. MicroRNA

MiRNAs are key regulators of gene expression. By binding to multiple genes at once, they create a complex network of post-transcriptional fine co-regulations.

miR-6728: has been demonstrated to be able to regulate several genes associated with cardiometabolic phenotypes [59].

miR-639: recently emerged as potential novel biomarker of Human bone marrow mesenchymal stem cells aging [60].

miR-661: has been demonstrated to have a regulatory role in small GTPase signalling. It also has an active role in phosphorylation of myosin II and up regulates the cell invasion, indicating a possible oncogenic miRNA [61]. *miR-661* is also highly associated with nano-sized vesicles released by activated *CD4+* T lymphocytes release [62] showing a correlation with inflammatory response, and with the modulation of the endogenous levels of *iNOS* and nitrite production via *MTA1* mediated control. [63]

miR-1248: studies investigating the relationships between miRNA profiles and aging reported that serum expression levels of *miR-1248* were significantly lower in old participants (mean age, 64 years) than in young participants (mean age, 30 years). *miR-1248* was found to regulate the expression of mRNAs related to several cytokines (age-associated cytokines *IL6* and *IL8*) and inflammatory-associated pathways (including NF-kB), thus suggesting a relevant role in the aging process. *miR-1248* also influences the impaired DNA repair capacity, then decreased levels of *miR-1248* due to aging directly affect DNA repair pathways [64].

miR-208a: targets thyroid hormone receptor associated protein 1 (*THRAP1*) and myostatin, which are negative regulators of muscle growth and hypertrophy. It is particularly relevant in cardiac myocytes, since its transgenic overexpression in the heart is sufficient to induce hypertrophic growth and arrhythmias in mice. *miR-208a* also has a key role in the expression of cardiac transcription factors, like homeodomain-only protein (*HOP*) and *GATA4*, and for the gap junction

protein connexin. Upregulation of *miR-208a* was observed in samples of infarcted heart tissue from patients with myocardial infarction compared to healthy adult hearts. Given its high sensitivity in early setting of acute myocardial infarction (AMI), *miR-208a* seems a reliable biomarker for early AMI diagnosis in humans [65]

F. Transcription Factors

TP53: TP53 encodes a tumor suppressor protein, whose expression is stabilized by DNA damage. In order to suppress tumor development, *TP53* has a key role in leading to senescence as mechanism to prevent tumor formation and to have a trade-off relation with cancer. Overexpression of *TP53*, in fact, induces cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism [36].

NFKB1: is a binding subunit of the *NFKB* protein complex, a transcription regulator that acts as hub by aggregating multiple intra- and extra-cellular stimuli (i.e., cytokines, oxidant-free radicals, ultraviolet irradiation, pathogens). Ectopic activation of NFKB has been associated with inflammatory diseases and inappropriate immune cell development or delayed cell growth. In particular, the *NFKB1* subunit is known to be responsible for proinflammatory arterial phenotype developed with aging, and is associated to vascular dysfunctions due to impaired nitric oxide processing [66].

MYC: is a multi-functional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. *MYC* is necessary and sufficient for the regulation of Nucleostemin (*NS*) (a nucleolar protein regulating stem cell proliferation and pluripotency). *NS* regulation is essential to preserve the regenerative potential of aging stem cells by antagonizing senescence and by enhancing myocardial regeneration. Levels of *MYC*, positive transcriptional regulators of the cell cycle, are decreased in myocardial senescence along with elevated levels of *TP53*, which eventually result in enlarged myocytes, reduced number of cardiomyocytes, disfigured mitochondrial morphology, telomere attrition, up-regulation of molecular markers of senescence, and decline in cardiac function resembling a heart failure phenotype [67].

IV. Conclusions

This paper presented a pure computationally inferred model that is able to represent the relationship between key genes and miRNAs involved in cardiac senescence processes. Interestingly, all the newly introduced regulators, except for two miRNAs (i.e., miR-4691 and miR-7133), appear strongly related to previous studies on aging related mechanisms. Giving the results obtained on the other regulators, these two miRNAs represent good candidate for further studies to elucidate their possible involvement in cardiac aging. On-going work is now focusing on laboratory experiments to validate some of the most promising interactions identified in the model.

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