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Mechanosensing dysregulation in the fibroblast: a hallmark of the aging heart

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Abstract

The myofibroblast is a specialized fibroblast that expresses α -smooth muscle actin (α -SMA) and participates in wound contraction and fibrosis. The fibroblast to myofibroblast transition depends on chemical and mechanical signals. A fibroblast senses the changes in the environment (extracellular matrix (ECM)) and transduces these changes to the cytoskeleton and the nucleus, resulting in activation or inhibition of α -SMA transcription in a process called mechanosensing. A stiff matrix greatly facilitates the transition from fibroblast to myofibroblast, and although the aging heart is much stiffer than the young one, the aging fibroblast has difficulties in transitioning into the contractile phenotype. This suggests that the events occurring downstream of the matrix, such as activation or changes in expression levels of various proteins participating in mechanotransduction can negatively alter the ability of the aging fibroblast to become a myofibroblast. In this review, we will discuss in detail the changes in ECM, receptors (integrin or non-integrin), focal adhesions, cytoskeleton, and transcription factors involved in mechanosensing that occur with aging.

Keywords

Cardiac fibroblast; myofibroblast; mechanosensing; aging

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Conflict of Interest

None.

1. Introduction

The myofibroblast, a specialized fibroblast that expresses the contractile protein α -smooth muscle actin (α -SMA), appears in the myocardium upon injury. In fact, in the uninjured young heart, there are very few myofibroblasts present (mostly around arterioles) (Shinde et al., 2017). Myofibroblasts appear in the heart after injury and play a distinct role depending on the type of injury; they are indispensable for proper scar formation and contraction after myocardial infarction (Hinz, 2006) but in other types of cardiac injuries (pressure overload) they participate in adverse remodeling (Moore-Morris et al., 2014). The transition from the fibroblast into myofibroblast depends on chemical and mechanical signals (Czubryt, 2019), but the distinction in vivo between the contribution of these two classes of signals is challenging. The major chemical activator is TGF- β , and it is released from its latent form upon demand (Buscemi et al., 2011; Klingberg et al., 2014). Mechanical signals often come from changes in protein composition of extracellular matrix (ECM) (see Table 1 for abbreviations) that translates to either increased tension and/or altered ECM composition. Changes in matrix very often are accompanied by an altered expression of different components, or activation of various receptors that connect cells with the ECM, such as integrins or transient receptor potential (TRP) channels. These mechanical signals translate into rearrangements and activation of various proteins that form focal adhesions (FA), a functional bridge between ECM and cytoskeleton. The cytoskeleton then undergoes a transformation, and a key functional protein, actin, becomes polymerized (F-actin). Polymerized actin then facilitates Myocardin-Related Transcription Factor A (MRTF-A) translocation to the nucleus where it can pair with another transcription factor, Serum Response Factor (SRF). Each factor binds to its cognate site on the α -SMA promoter, leading to its transcriptional activation.

In the past, we examined the role of TGF- β in myofibroblast transdifferentiation in aged hearts (Trial and Cieslik, 2018). We have found defects in the canonical TGF- β Smad-dependent pathway (Cieslik et al., 2011b). Specifically, we found that the fibroblasts derived from the aging hearts had reduced responsiveness to TGF- β , which translated to a weaker α -SMA expression in vitro and reduced myofibroblast numbers after myocardial infarction in vivo, as noted by others (Bujak et al., 2008). This defect may cause reduced tensile strength of the scar, altered heart geometry and ultimately leads to increased post-infarction remodeling. In this review, we concentrated our efforts on understanding how mechanical signaling and mechanosensing are altered in the aged cardiac fibroblasts. The idea for this review originated from our observation that even without chemical signaling (TGF- β), in vitro α -SMA expression in the fibroblasts isolated from the aged hearts was reduced when compared with the ones derived from the young hearts, suggesting impaired mechanosensing (Cieslik et al., 2011b). Most studies related to the topic are based on observations from various cardiac injury models in young rodents. However, it became apparent that the multiple signaling and structural changes that we observe in the aging heart or fibroblasts derived from the old heart contribute to the alternative mechanosensing that translates into the reduced fibroblast-to-myofibroblast transition (Figure 1). This review attempts to depict the role of these effectors and to provide insight into their potential interactions.

2. The cardiac fibroblast and its role in cardiovascular diseases

The primary fibroblast function in the healthy and diseased heart is synthesis, deposition, and crosslinking of the matrix. In disease, this is characterized by excessive ECM deposition and crosslinking, leading to fibrosis. Fibrosis manifests with nearly all forms of heart disease, and its progression is clinically linked to heart failure. Cardiac fibrosis can develop in aging, aortic stenosis, hypertension, cardiomyopathies, and coronary heart disease without infarction (Hinderer and Schenke-Layland, 2019; Kong et al., 2014) or as a result of a poorly formed scar after myocardial infarction in the old (Bujak et al., 2008) or diabetic heart (Thakker et al., 2006); however, the prevalence for these diseases increases with aging.

There are no diagnostic assays or tests that will directly evaluate fibroblast function in vivo. The only possibility to have an insight into fibroblast “health” is to detect and quantify fibrosis. This can be achieved via invasive and non-invasive methods. For the direct method, the collagen level evaluation in the endomyocardial biopsy is the only method currently available (Hinderer and Schenke-Layland, 2019). The non-invasive imaging methods include magnetic resonance imaging, integrated backscatter echocardiography, single-photon emission tomography, and positron emission tomography. Finally, there is also a possibility to assess the levels of circulating carboxy-terminal pro-peptide of collagen type I that reflects on the level of newly synthesized collagen, but this method does not identify which organ is undergoing fibrotic changes (Hinderer and Schenke-Layland, 2019).

The pathological activation of fibroblasts that contribute to fibrosis can be curbed using several different approaches, such as angiotensin-converting enzyme blockers, angiotensin receptor 1 antagonist, beta-blockers, endothelin antagonists, statins, cyclic GMP-specific phosphodiesterase type 5A inhibitor, and loop diuretics (Fang et al., 2017). Most of these available treatments directly affect collagen synthesis, but statins work by quenching inflammation. It has been shown that inflammation can promote fibrosis (Trial et al., 2017).

As the heart ages, fibroblasts start to undergo phenotypic changes. They become activated, which leads to matrix deposition and crosslinking and they start to secrete various cytokines, switching to a pro-inflammatory phenotype (Cieslik et al., 2015). It is not known if the entire fibroblast population expands with aging or if any sub-population is preferentially activated. Fibroblast heterogeneity has been extensively studied in various injury mouse models in young animals (Tallquist and Molkenin, 2017), but not much has been reported in regards to aged animals. Nevertheless, it has been demonstrated that the aging heart displays increased interstitial and adventitial fibrosis (Alex et al., 2018), suggesting that at least two different populations are activated; PDGFR α ⁺ (Moore-Morris et al., 2014) and Gli⁺ fibroblasts (Kramann et al., 2015). The former is associated with interstitial and the latter with adventitial fibrosis. Our group previously reported an increased number of activated fibroblasts in the aged myocardium (Cieslik et al., 2013b); however, we did not identify the sub-population. Interestingly, the number of α -SMA⁺ cells is not increased with aging (in the uninjured heart) (Alex et al., 2018), although after myocardial infarction, their number is reduced when compared with the young heart (Bujak et al., 2008) suggesting an impaired myofibroblast differentiation program. Recent studies have also demonstrated that cardiac and dermal fibroblasts from old male and/or female mouse hearts have a higher

transcriptional heterogeneity (Ali et al., 2014; Farbehi et al., 2019; Mahmoudi et al., 2019; Vidal et al., 2019). The reason for this heterogeneity remains unclear since it could be due to mixed origins of resident fibroblasts in aged hearts, or to the accumulation of different events leading to transcriptional (and epigenetic) drift between distinct fibroblast populations. Yet, the consequences are obvious, since it means that both ECM synthesis and applied mechanosensing may differ from one fibroblast population to another, with young-like versus aged islets of cells and secreted matrix, leading to an unsynchronized response to mechanical stress.

3. Extracellular matrix

The ECM forms a mechanical scaffold necessary to maintain the geometry of organs. The ECM also plays a significant role in signal transduction regulating cellular function. The activated fibroblast/myofibroblast is the primary ECM protein producer (Hortells et al., 2019). Because all cardiac cells can influence each other via secreted factors (Frangogiannis, 2019) or through coupling (MacCannell et al., 2007), the changes in ECM directly or indirectly can affect all cells residing in the interstitium and may result in changes in heart function, including heart failure.

The major differences in ECM in the aging heart (when compared with the young heart) are the expansion of the interstitial and perivascular ECM repository, increased collagen cross-linking, or deposition of different isoforms of fibronectin (FN) (Cieslik et al., 2017; Gazoti Debessa et al., 2001).

The main components of the cardiac ECM collagen network are fibrillar collagen I and III (Weber et al., 1988), that are best characterized in various pathologies, aging included. Since there is a noticeable discrepancy between mRNA and protein levels of collagens in the old heart, we will discuss here only changes related to protein levels. Data from the human and mouse heart demonstrate increased levels of collagen type I (Cieslik et al., 2011a; Gazoti Debessa et al., 2001). These data are also supported by in vitro observations wherein aged fibroblasts synthesize twice as much procollagen type I as the young ones (Cieslik et al., 2013b), consistent with reports showing that the total collagen content in the old male and female heart doubles when compared with the young heart (Eghbali et al., 1989; Lin et al., 2008). Data from human hearts also point to the altered ratio between collagen I and III, with increased collagen I and reduced collagen III deposition (Mendes et al., 2012). The altered collagen I/III ratio may impair left ventricle function via increased stiffness as shown in both sexes (Wei et al., 1999). Cross-linking of collagen can occur either through an enzymatic process (via lysyl oxidase) or via a non-enzymatic reaction (between glucose and lysine or hydroxylysine residues leading to the formation of advanced glycation end products). Both enzymatic and non-enzymatic cross-linking are augmented in the aging myocardium (Asif et al., 2000; Thomas et al., 1992), leading to the increased stiffness of the matrix.

The other collagens (non-fibrillar) or ECM matrix proteins also change with aging and can affect directly or indirectly the fibroblast phenotype and their transition into myofibroblasts. As we have described above, the matrix stiffness is increased in aging (Wei et al., 1999). The

presence of elastin very often compensates for collagen-related stiffness; however, it has been demonstrated that with age, its expression is reduced in human valves (McDonald et al., 2002), which may indicate similar changes in the ventricles. Interestingly, matrix stiffness by itself can increase α -SMA transcriptional activation in both males and females (Herum et al., 2017). However, fibroblasts derived from aged hearts (Cieslik et al., 2011b) or fibroblasts in injured old hearts (Cieslik et al., 2013a) have a reduced ability to become α -SMA-expressing myofibroblasts, suggesting additional or even multiple defects that contribute to this abnormality.

Aging also affects other important components that play various roles in the matrix. Biglycan and decorin, for example, have been shown to control myofibroblast transdifferentiation via downregulation of focal adhesions (Melchior-Becker et al., 2011) and expression of α -SMA (Melchior-Becker et al., 2011; Nakatani et al., 2008). Interestingly, the expression of these proteins is increased in the aged pig heart (Stephens et al., 2008). It has also been reported that certain glycoproteins such as periostin, tenascin-C, and thrombospondin-2 are upregulated in the aged heart or fibroblasts derived from the old heart (Li et al., 2014; Sato and Shimada, 2001; Swinnen et al., 2009). None of these proteins directly affects α -SMA transcriptional regulation; however, periostin may have an indirect effect because it affects ECM homeostasis by interacting with collagen I, FN, tenascin-C, and heparin (Norris et al., 2007).

Finally, FN and its variants play a role in myofibroblast differentiation. FN serves as a “bridge” between cells and other ECM proteins by binding to cell membrane receptors and ECM proteins (collagens, fibrillin, tenascin-C, heparin) (Chung and Erickson, 1997; Kadler et al., 2008; Sabatier et al., 2009). After stress or injury, there are several splice variants of FN that are transcribed with extra domain A (FN-EDA) or extra domain B, but specifically, the presence of FN-EDA has been attributed to an increased fibroblast to myofibroblast transition (Kohan et al., 2010). Interestingly, in the aging uninjured heart, there is FN-EDA (Cieslik et al., 2017), but myofibroblasts were not detected in the interstitium of the male or female heart (Alex et al., 2018). Increased stretching of FN fibrils can lead to the partial unfolding of the secondary structure and change of binding site availability (Antia et al., 2008), which may explain why in the aging heart, even in the presence of FN-EDA, there are very few myofibroblasts.

4. Mechanosensing at the membrane

4.1 Integrin-mediated mechanosensing

Mechanosensing from the ECM to the cytoplasm of the fibroblasts is directed through transmembrane adhesion molecules, many of which are integrins (Giancotti and Ruoslahti, 1999). Integrins are heterodimers, with α and β chains (Figure 2). The class of integrins that are able to bind to the ECM are mainly β 1 coupled to a variety of α chains, which have the specificity to allow binding to various ECM components such as collagen, fibronectin, or laminin. Integrins and other adhesion molecules are essential mediators of fibroblast functions such as migration and contractility (Figure 2), which are diminished in aged cells (Cieslik et al., 2011b). It is, therefore, likely that reduced mechanosensing by these cells could be responsible for the compromised functions. Although the expression levels of

various α and β integrin chains have been reported to change in aged animals (reviewed in (Meschiari et al., 2017)), the effect of these changes on the functions of the cells cannot be determined because of the complex nature of the α - β pairing (availability of each chain for assembly) and their subsequent activation to allow binding to the ECM.

Integrins signal to the cell bidirectionally, called “outside-in” or “inside-out”, depending on whether the originating signal comes from the external matrix or from focal adhesion molecules bound to the integrin cytoplasmic tail within the cytoplasm. The signaling may also be simultaneous. Signaling and increased binding to ligands is accomplished by a conformational change in the heterodimer, including the change from a bent to an open configuration (reviewed in (Liu et al., 2015b)). Whereas the integrins of most hematopoietic cells are in the “resting” configuration to prevent their adhesiveness while in the blood, fibroblasts have some of their integrins in the active state because of their requirement for anchorage in a tissue (Norris et al., 2007). No comparison of the integrins in the active versus resting states has been done in young versus old cardiac fibroblasts.

Outside-in signaling is accomplished by integration of mechanical and biochemical forces. Integrins respond to properties of the ECM and the tissue in which it resides: force, rigidity, and the spatial arrangement of the ECM (Kechagia et al., 2019). Changes in the heart occur with aging, so that there is an increase in stiffness (rigidity) and the appearance of new matrix proteins such as FN-EDA that may affect the ECM spatial arrangement (Cieslik et al., 2017). The added stiffness may also be a result of matrix cross-linking (Meschiari et al., 2017). These may result in forces that change the conformation of the integrin to its fully active state, thus increasing its affinity for matrix. Further, the changes can have a “memory”, in that they can persist after the force has been removed (Kechagia et al., 2019). Inside-out signaling may derive from cellular responses to cytokines and chemokines (Gahmberg et al., 2019), some of which are markedly increased in the aging heart (Cieslik et al., 2011a; Cieslik et al., 2015), or from other stimuli. The fibroblast then accumulates molecules of the focal adhesion at the site of the integrin cytoplasmic tail, and the integrin assumes its activated conformation.

4.2 Non-integrin mechanosensing

Cardiac fibroblasts also express TRP channels, which are members of a superfamily of molecules that sense an extraordinary range of environmental changes, including pH, osmolarity, temperature, and chemical (reviewed in (Venkatachalam and Montell, 2007)). One of the most characterized for its induction by mechanical signals is TRP vanilloid 4 (TRPV4). It is a calcium channel that can be activated when a force is applied to β 1 integrins; this force is transduced by a mechanical strain in the cytoskeletal backbone of the focal adhesions associated with the integrins (Matthews et al., 2010). In addition to this indirect mechanism for mechanosensing, direct activation of a calcium influx can be induced by shear stress, stretch, and many other stimuli (Michalick and Kuebler, 2020; Zhan and Li, 2018). TRPV4 activation has been linked to fibrosis in many tissues (Zhan and Li, 2018). This may be because differentiation of fibroblasts into myofibroblasts is dependent on non-canonical TGF- β signaling in association with TRPV4, which responds to increased ECM stiffness (Adapala et al., 2013). The latter study of cardiac fibroblasts reported that

mechanical stiffness transduced via TRPV4 resulted in signaling through focal adhesions, including Rho-GTPases (Adapala et al., 2013). In lung fibroblasts, signaling through TRPV4 facilitates actomyosin remodeling, thereby causing nuclear translocation of the Myocardin-Related Transcription Factor-A (MRTF-A) to enhance α -SMA transcription (Rahaman et al., 2014). It has been suggested that this pathway might be operative in the cardiac ventricle (Inoue et al., 2019). Although little is known about changes in TRPV4 expression in aging fibroblasts, a noted increase of TRPV4 expression in aged cardiomyocytes is associated with hypercontractility and a decline of cardiac function (Jones et al., 2019). Responsiveness to the known increase in matrix stiffness with aging may be the link between TRPV4 as a mechanosensor and fibrosis.

5. Focal adhesions

Focal adhesions (FAs) represent multi-unit structures that are organized around active integrins at the plasma membrane to bridge the ECM to the inner cytoskeleton (Burrige, 2017) (Figure 2). By transducing ECM mechanical tension into biochemical cell signaling and mechanical inner force, FAs thus play a key role in cell motility, proliferation, adhesion and migration. The size of FAs can predict cell migration capacity in both mouse embryonic and human male skin fibroblasts during wound healing (Kim and Wirtz, 2013). Due to the multiple functions of FAs, the integrin-actin protein binding complex (the so-called integrin “adhesome”) includes a wide variety of kinases, actin-binding proteins and adapters, for more than 180 dynamic and context-actionable protein-protein interactions (Horton et al., 2016; Horton et al., 2015; Zaidel-Bar and Geiger, 2010). Therefore, its composition and role in the aging cardiac fibroblast is both complex and multi-layered. Critical FA core proteins are shown in Figure 2.

5.1 Talin and vinculin

Talin is an actin-binding protein that is indispensable and sufficient for integrin engagement in FAs (Chinthalapudi et al., 2018). It interacts with the integrin β -chain via its “head” FERM domain (Protein 4.1- Ezrin- Radixin- Moesin), while its “tail” domain binds to a polymerized actin filament (F-actin) and to vinculin, an actin-binding protein that interacts with F-actin and also with α -actinin, another actin-binding protein promoting F-actin parallel cross-linkage (Figure 2) (Klapholz and Brown, 2017). Main adaptors of the integrin adhesome are kindlins and paxillin (Figure 2). Neither possesses enzymatic activity by itself, but both rather promote the recruitment of kinases and phosphatases, which locally initiates intracellular signaling in response to mechanical forces. Vinculin levels are increased in the left ventricle in aging female rats and male rhesus monkeys, both at the inter-cardiomyocyte connection site (intercalated disk) and at the lateral border of these cells, which may thus include fibroblasts as well (Kaushik et al., 2015). Fruit flies overexpressing vinculin exhibited an increased lifespan and a delayed age-associated diastolic dysfunction (Kaushik et al., 2015). Therefore, the increase in vinculin in the aging mammal heart probably represents an adaptive response to tension at the FA site and downstream gene expression, but its beneficial effect may be eventually overwhelmed due to accumulated changes along the mechanosensing axis.

5.2 Kindlins and paxillin

Kindlins represent a family of conserved FERM-domain proteins that are recently described as central integrin regulators. The three family members have a tissue-restricted distribution, while holding specific binding partners: while kindlin-1 is more ubiquitous, kindlin-3 is restricted to hematopoietic cells (podosomes) (Rognoni et al., 2016). No disease-causing mutation is reported for kindlin-2, but a knock-out is lethal in the mouse due to cardiovascular defects (Dowling et al., 2008a; Montanez et al., 2008). Kindlin-2 is the most predominant in cardiac cells, including in fibroblasts, which may indicate a key role in myocardial tissue integrity during both development and post-natal maintenance (Dowling et al., 2008a; Dowling et al., 2008b; Zhan et al., 2014). Conditional knock-out in the adult mouse leads to dilated cardiomyopathy characterized by invading and massive fibrosis (Zhang et al., 2016), while shRNA against kindlin-2 reduces fibroblast mechanical strength and myofibroblast differentiation (He et al., 2011). According to biochemical studies, kindlin-2 works as a dimer, and any impairment of kindlin-2 dimerization affects cell adhesion (Li et al., 2017b). Interestingly, kindlin-1 and kindlin-2 have a specific affinity to each other, which may be due to high sequence homology among the kindlin protein family. But it might also suggest that, in several cell types in which kindlins-1 and -2 are co-expressed, kindlin-1/kindlin-2 heterodimers could be generated with potential downstream consequences in terms of FA stabilization and mechanosensing. Regular kindlin-2 partners include not only $\beta 1$, $\beta 2$, and $\beta 3$ integrin cytosolic tails (Bledzka et al., 2010; Bouaouina et al., 2012; Li et al., 2017b; Liu et al., 2011), but also Integrin-linked Kinase and migfilin (Brahme et al., 2013), both involved in integrin signaling and actin cytoskeleton coupling (Fukuda et al., 2014) (Figure 2). Integrin-linked Kinase has been notably reported as a lifespan regulator (Kumsta et al., 2014), and its overactivation in young animals mimics aging-like cardiac defects (Nishimura et al., 2014). Sos (Son of sevenless) and Rac1 GTPase are two additional kindlin-2 binding proteins that can trigger the formation of the actin cytoskeleton (Jung et al., 2011; Wei et al., 2014). Kindlins work side-by-side with paxillin, another important FA scaffolding protein (Theodosiou et al., 2016).

Due to the presence of many phosphorylatable Ser/Thr and some tyrosine residues, paxillin plays a central yet dual role, since it can facilitate both FA assembling and dissociation in response to an interplay of kinases and phosphatases (including ERKs, JNK, PKA, PKC and GSK3) (Lopez-Colome et al., 2017; Mekhdjian et al., 2017; Zhou et al., 2017). As an adapter, paxillin recruits and indirectly favors the activation of Focal Adhesion Kinase (FAK) (Figure 2). Interestingly, the reduction of paxillin expression observed in dermal fibroblasts with aging resulted in fibroblasts is characterized by an abnormal phenotype and spreading pattern. Furthermore, inhibition of paxillin expression resulted in diminished myofibroblast transition, suggesting that this protein plays a central role between FA and cytoskeleton rearrangements leading to α -SMA expression (Zheng et al., 2012).

5.3 FAK

FAK is the main driver of mechano-induced cell signaling and is indispensable for fibroblast function (Gilmore and Romer, 1996). FAK has multiple abilities within a cell, including acting as a scaffolding protein complex, a bridge and/or as a tyrosine kinase (Ceccarelli et al., 2006; Hayashi et al., 2002; Nowakowski et al., 2002). FAK binds to integrins via its

FERM domain, while its Focal Adhesion Targeting domain binds to paxillin, forming a bridge between the cytoskeleton and ECM receptors (Figure 2) (Hayashi et al., 2002). FAK membrane recruitment at the FA site promotes mechanical-induced conformational changes of FAK dimers, triggering its autophosphorylation, which in turn may favor the binding of Src and the consequent activation of FAK resulting in an active FAK/Src complex (Wu et al., 2015). Beyond the FA site, the FAK has an affinity for other growth factors or cytokine receptors (e.g.: Epidermal Growth Factor Receptor) that can reinforce FAK activity or promote its degradation (Chen and Chen, 2006; Sieg et al., 2000). In addition, phosphatidylinositol 4,5-bisphosphate has a higher avidity for FAK dimers (Brami-Cherrier et al., 2014), so FA sites become enriched in this phospholipid, providing substrates for actin polymerization and FAK-associated downstream cell signaling (Figure 2) (Goni et al., 2014). Therefore, FAK aberrant transduction might have a role in cardiac fibroblast aging; that is, the source of the aging defect might be on the FA dock (talin/paxillin/kindlin) or via Src recruitment (Figure 2).

5.4 Mechanosensing mechanism in fibroblasts

In female human lung fibroblasts, mechanical cell signaling, and activation of gene expression have been described as FAK-dependent through PI3K/AKT/mTOR mobilization (Xia et al., 2004). Mechanically induced biochemical responses driven by FAK include some GTPases (Rho/MDia, Rac, cdc42) and actin-binding proteins (N-WASP, Arp2/3), which regulates microtubules and actin cytoskeletal assembling/disassembling dynamics (Figure 2) (Romero et al., 2020; Shen et al., 2013). The myogenic transcriptional factor Myocyte Enhancer Factor 2a is also reported to be downstream from FAK activation (Peng et al., 2008). Although this specific FAK/Myocyte Enhancer Factor 2a axis might be disputable in fibroblast mechanosensing, other phosphorylation-dependent transcription or co-transcription factors involved in myofibroblast gene programs will follow this path, as will be discussed later in the nucleus section. Finally, MAP kinases, especially JNK and ERKs, represent main downstream effectors of FAK (Figure 2). JNK in turn, activates YAP/TAZ, SMAD4, and NFAT4, thus favoring cell survival, differentiation, and motility, and therefore the myofibroblast gene program (Zeke et al., 2016), as will be discussed below. Cardiac fibroblasts derived from the old heart have reduced activated JNK levels when compared with fibroblasts derived from the young mouse heart, supporting the notion that the FAK-JNK pathway is defective in the aging heart (Cieslik et al., 2011b).

In conclusion, FA ensures the connection between the ECM and the actin cytoskeleton, thus representing the main initiator of the intracellular signaling in response to ECM tension. As ECM composition changes in the aging heart, FA activity and assembly may be affected downstream. Yet, currently, only a few clues have been accumulated. For instance, an increase of vinculin, which bridges FA to the actin filament, seems to take part in an aging-remodeling process, while a decrease of paxillin is reported in aged skin fibroblasts. The altered JNK pathway in aging cells may support the concept of an affected FA signaling in aging cardiac fibroblasts, which requires further investigations. Considering the numbers of proteins within the integrone, the impact of FA is probably underestimated.

6. Cytoskeleton mechanotransduction

6.1 Actin, as a part of the cytoskeleton

The cytoskeleton rearrangement represents the mechanical response to ECM tension. In fibroblasts, this backbone includes a more-or-less dense meshwork of F-actin (also called microfilaments), a connecting network of microtubules and a polarizing frame of intermediate filaments of vimentin (Grabec et al., 2000; Ochsner et al., 2010). This specialized cytoskeleton thus provides mobility to the fibroblasts and contractile abilities to the mature myofibroblasts during wound resolution. Although vimentin is mentioned in a few cases of invading fibrosis, such as in idiopathic pulmonary fibrosis (Li et al., 2017a), and in aging human female dermal fibroblasts (Sliogeryte and Gavara, 2019), the mechanotransduction in cardiac fibroblasts involves mostly the actin cytoskeleton, on which microtubules play a supportive role (Omelchenko et al., 2002). Fibroblasts have a reduced ability to differentiate into myofibroblasts during aging, while their cytoskeleton exhibits a network of F-actin that is impaired (Table 2) (Cieslik et al., 2011b). Consequently, the deterioration of the actin cytoskeleton may be a part of the aging process in cardiac fibroblasts. Age-related changes can affect not only the actin dynamic treadmill but also actin(s) expression itself, as we have demonstrated before (Table 2) (Cieslik et al., 2011b) and both can apply a regulatory feedback to ECM, integrin and receptor signaling and gene transcriptions that can exacerbate age-related defects.

6.2 Actin in fibroblast maturation

The myofibroblast ensures tissue integrity and efficient healing processes, due to its ability to apply contractile force to a surface, as demonstrated in lung and cardiac fibroblasts from both male and female rats (Hinz, 2006; Santiago et al., 2010). Aside from the application of isometric force (Gardel et al., 2008), myofibroblasts exhibit a dense contractile apparatus that differs from the migratory structures. In fact, cytoplasmic β - and γ -actin isoforms are the ones involved in cell morphology and motility, whereas the myofibroblast cytoskeleton is highly enriched in α -SMA (Hinz et al., 2001; Hinz et al., 2003), an alpha-isoform that is usually found in contractile muscle cells such as striated myocytes (Clement et al., 2007) and smooth muscle cells (Chamley et al., 1977) in both male and female tissues. Despite extensive studies from the 1970s, it remains unclear whether α -SMA is the exclusive actin isoform within the myofibroblast contractile apparatus or if it co-polymerizes with β -/ γ -actins. Yet, the importance of α -SMA in myofibroblast contractile force is reported both in vivo and in vitro: notably, the induction of Acta2 gene expression (encoding α -SMA) is enough to dramatically increase the traction force of the myofibroblast (by 2 to 3-fold compared with the isometric tension applied to quiescent cells) in cells originated from both male and female rats (Chen et al., 2007; Goffin et al., 2006; Hinz et al., 2001; Hinz et al., 2003). In the aging male mouse heart, the decrease in α -SMA level is one of the main aging features we discovered in cardiac fibroblasts (Table 2), which is paired with a lower amount of and disorganized F-actin network and consequently impaired contractility on a collagen matrix in vitro (Cieslik et al., 2011b).

6.3 The cytoskeleton of aging cells

Globally, aging impairs actin polymerization due to upstream changes in cell signaling (from FA and beyond) and local decrease in ATP availability, as well as downstream protein misfolding and a decrease in protein homeostatic expression (Lai and Wong, 2020). Additionally, oxidative stress can depolymerize oxidized actin, by a non-enzymatic process, leading to F-actin crosslinking and G-actin misfolding (Farah et al., 2011; Varland et al., 2019), but also by the catalytic activity of Molecule Interacting with CasL, a methionine oxygenase, and Methionine-R-sulfoxide reductases (Aberle, 2013). By these redox enzymes, actin can thus be sulfoxidized, which promotes its depolymerization and prevents new filament formation (Giridharan and Caplan, 2014; Grintsevich et al., 2017; Hung et al., 2011; Ilani and Fass, 2013). The activity of these Methionine-R-sulfoxide oxygenases and reductases is known to be involved both in aging (Lourenco Dos Santos et al., 2018) and in fibroblast and macrophage actin homeostasis (Giridharan and Caplan, 2014; Lee et al., 2013), but there is no current link established in the specific case of aging cardiac fibroblasts. However, oxidative stress is elevated in cardiac fibroblasts derived from aged hearts (Cieslik et al., 2014) and a similar mechanism has been recently demonstrated in cardiomyocytes (Angelini et al., 2020). Considering the high conservation of actin function, we hypothesize that an oxidative stress/actin double axis might also be involved in the dysfunction of aging cardiac fibroblasts. This is the reason why redox-signaling and metabolic function gauged by AMP Kinase (Gu et al., 2018) are foreseen as potential target(s) in injury-caused deleterious myofibroblast differentiation (Sampson et al., 2012). But, in the aging heart, since myofibroblast transition is impaired (rather than intensively triggered), this kind of drug therapy may not be helpful. In human skin fibroblasts (from both sexes), aging-mimicking artificial F-actin disorganization rapidly decreases TGF β -RII in those cells, leading to a consequent reduction of collagen expression (Qin et al., 2018). We have found in the aging fibroblasts of the male mouse heart a reduced expression of both TGF- β receptors and disorganized F-actin (Cieslik et al., 2011b), which may suggest additional roles of actin.

6.4 Actin and mRNA “zip-coding”

The role of actin extends beyond the cytoskeleton and it includes transcriptional and post-transcriptional activities that may be equally affected by aging. Actin is notably involved in the mRNA “zip-coding”, a recently described mechanism that regulates the subcellular localization and translation of mRNAs (Jansen, 2001; Lopez de Heredia and Jansen, 2004). First, F-actin binds to zip-code-binding protein 1 (ZBP-1), a highly-conserved RNA-binding protein that recognizes a specific motif in the 3'UTR of these “zip-coded” mRNAs and ensures their mobility through an actin polymerization pushing force (Nicastro et al., 2017). Interestingly, fibroblasts represent one of the few cell types in which such a mechanism persists after embryogenesis, which may suggest an essential role in its specific function. Notably, β -actin mRNA is one of the most extensively zip-coded mRNAs and it is specifically enriched at the FA site during lamellipodia formation and directionality, but also along actin stress fibers (Bassell et al., 1998; Kislauskis et al., 1994; Shestakova et al., 2001). During the past few years, clear feedback has been demonstrated between integrin signaling, F-actin and mRNA zip-coding in fibroblasts, which could suggest mRNA zip-coding to be a concrete part of the mechanosensing in this cell (Chicurel et al., 1998). While

integrin signaling promotes ZBP-1 phosphorylation by Src and so its further activation, β -actin mRNA zip-coding is ZBP-1/F-actin-dependent, and both processes enhance FA assembling and fibroblast mobility (Chicurel et al., 1998; Huttelmaier et al., 2005; Katz et al., 2012). For years, mRNA processing has been suspected to be affected by aging (as reviewed in (Borboldis and Syntichaki, 2015)); therefore, impairment of mRNA zip-coding may be involved during aging (Table 2).

6.5 Actin at the core of the transcription machinery

Apart from its role in the cytoplasmic sorting of mRNA, actin can also be engaged in nuclear transcription. It has been demonstrated that nuclear actin can associate with all the three RNA polymerases (I, II and III) and is thus necessary for RNA transcription (Table 2) (Fomproix and Percipalle, 2004; Hu et al., 2004; Percipalle et al., 2003; Philimonenko et al., 2004; Serebryanny et al., 2016; Visa, 2005). Therefore, the impairment of actin turnover in aging cardiac fibroblasts may also affect the activity of these different RNA polymerases, hence the whole gene expression machinery, although the mechanism has not been well explored. The role of actin in gene expression is not limited to its docking function for RNA polymerases (Table 2). In fact, it is also well-known that the actin treadmill takes an active part in the regulation of the SRF/MRTFA axis, as will be described below.

Actin represents the mechanical core of the cardiac fibroblast, ensuring both cellular function and signaling (Table 2). During the myofibroblast maturation and healing process, the actin cytoskeleton transits to a network of α -SMA enriched contractile fibers, and we previously demonstrated that this ability is specifically impaired in cardiac fibroblasts of the aging heart. In addition, albeit no direct link has been established in the specific case of aging cardiac fibroblasts, many features of aging, such as oxidative stress, are known to have an impact on the actin treadmill (F-/G-actin imbalance) (Table 2). The role of actin is extremely complex within the cells since it can be involved in RNA polymerases complex formation, but also in gene transcription and both are known to be affected in aging.

7. Nucleus and gene transcription

Myofibroblast gene program transcription is the ultimate step of mechanosensing. In fibroblasts from the aging heart, the alterations that are accumulated from mechanosensing lead to a decrease in α -SMA expression and extensive changes in ECM production. Therefore, a combination of alterations within different transcriptional axes may be involved. Since many of them are interconnected, the story in the nucleus is complex and remains elusive for some parts. Here we will focus on the ones that are important for mechanosensing and aging, such as Serum Response Factor (SRF), Myocardin-Related Transcription Factor-A (MRTF-A), Yes-associated protein (Yap) and Transcriptional co-Activator with a PDZ motif (Taz) (as summarized in Figure 3 and Table 3). Several age-related epigenetic and post-transcriptional modifications will also be discussed below.

7.1 SRF/MRTF-A

SRF/MRTF-A represents a major transcriptional axis that translates mechanosensing into gene expression, triggering multiple possible cell fates from survival to differentiation and

migration. SRF is a ubiquitous and highly conserved transcription factor that affects an extensive list of more than 8000 target genes, including its own gene as a main target (Khanday et al., 2016; Sun et al., 2006). MRTF-A is an SRF co-factor that is actin-regulated (Miralles et al., 2003; Posern et al., 2004; Posern et al., 2002). In resting cells, monomers of actin (G-actin) bind to MRTF-A, leading to MRTF-A cytosolic retention, while mechanical or biochemical stimuli (either ECM tension or growth factor signaling) promote F-actin formation, and thus the release of MRTF-A from G-actin, finally promoting its nuclear translocation and local binding to the SRF dimer (Figure 3). Altogether, the SRF/MRTF-A axis notably regulates the cytoskeleton gene program, including the expression of all the actin isoforms (from β -/ γ - to α -SMA) (Medjkane et al., 2009; Olson and Nordheim, 2010; Selvaraj and Prywes, 2004). Therefore, there is constant feedback between actin and SRF/MRTF-A. The preponderant role of SRF/MRTF-A in stress-induced myofibroblast differentiation is well-known and affected by RhoGTPase-associated actin dynamics (Esnault et al., 2014; Small, 2012). In addition, MRTF-A deficient adult mice develop reduced fibrosis and scar in response to isoproterenol stress or myocardial infarction (Small et al., 2010). In the literature, many studies have revealed the critical role of SRF (activity or protein level) in clinical cases and experimental models of cardiomyopathies (Chang et al., 2003; Davis et al., 2002), both leading to deleterious fibrosis (Gary-Bobo et al., 2005; Parlakian et al., 2005). The involvement of SRF during myocardial aging is also well documented, but currently, studies in the field have been mainly focused on whole-heart or cardiomyocyte-specific approaches. Therefore, the link between SRF/MRTF-A remains hypothetical in cardiac fibroblast aging. In the heart of old male and female rats, SRF basal protein level is greater than 2-fold increased, but it cannot be subsequently increased in response to myocardial infarction or pressure overload as it usually is in the young rat heart (Lu et al., 1998; Takahashi et al., 1992). This establishes a correlation between aging, maladaptive cardiac remodeling and SRF activity. In addition, mild overexpression of *Srf* gene in the male mouse heart, either total or cardiomyocyte-specific, leads to mild age-related features, including myocardial dysfunction and higher collagen deposition (Angelini et al., 2015; Zhang et al., 2003). Interestingly, the fact of artificially maintaining a youthful level of SRF in the heart of old mice trends to be beneficial against the long-term cardiac alterations due to isoproterenol stress (Azhar et al., 2007). Hence, SRF level and activity can be considered as a homeostatic gauge in cardiac cells. Noteworthy, SRF binding capacity to the promoters of its target genes is also affected during aging (Table 3) (Lu et al., 1998; Zhang et al., 2011). This specific alteration can be due to extensive defects in terms of cofactor availability or competition with other transcription factors. In fact, on the one hand, the inhibitory competition of co-factors (“squenching”) and MRTF-A retention are indeed reported (Lin et al., 2007; Prywes and Zhu, 1992). On the other hand, many SRF binding sites overlap with docking sites for other transcription factors (such as Yap/Taz or Nuclear factor of activated T-cells 4) that may thus compete for binding (Esnault et al., 2014; Sun et al., 2009), which could finally alter or even antagonize the global gene response. In the context of the aging heart, as previously mentioned, the defective mechanosensing leads to a higher rate of depolymerized actin, which may thus favor MRTF-A cytosolic retention, to the detriment of downstream SRF transcriptional balance. We hypothesize that age-related increased expression of SRF may be due to complex compensatory feedback, as an ultimate attempt to switch the transcriptional program back into an optimal adaptive range.

7.2 Yap/Taz

Paired with SRF/MRTF-A, Yap/Taz coactivators represent indispensable sensors and integrators of mechanosensing. A dual modulation exists for this dimer, through Hippo pathway-dependent or -independent pathways (extensively reviewed in (Ma et al., 2019; Piccolo et al., 2014) and (Totaro et al., 2018), respectively). Briefly, in resting cells, Yap/Taz can be phosphorylated by the Hippo pathway cascade of kinases (MST1/2, then LATS1/2), leading to their cytosolic retention and consequent degradation (Figure 3) (Ma et al., 2016). The absence of the Hippo pathway is thus reported to promote the nuclear translocation of the dimer in different cell lines (Zhang et al., 2017). But, as previously mentioned, Yap/Taz is also a substrate of FA downstream signaling via RhoGTPase (Rac1/p21 Activated Kinase) and Src, proteins that are both activated in response to FA formation and subsequent FAK activation (Lamar et al., 2019; Ma et al., 2015). However, since Rac1 and Src are also involved in F-actin formation and maintenance, Yap/Taz activation is thus equally dependent on cytoskeleton reorientation in response to ECM tension. In accordance with this concept, it has been demonstrated that a stiffer ECM, as it is developed in the aging heart, trends to promote Yap/Taz translocation, while a more elastic ECM favors their degradation (Figure 3) (Liu et al., 2015a). The involvement of Yap/Taz in myofibroblast differentiation is reported in the liver, skin and lung (reviewed in (Noguchi et al., 2018)), whereas a recent study suggests that increased activation of Yap/Taz promotes myofibroblast differentiation in a mouse model of dilated cardiomyopathy (Jin et al., 2018). Little is known about their involvement in aging cardiac fibroblasts, but recent reports suggest the importance of several complex crosstalks. First, Yap/Taz can facilitate the degradation of β -catenin or the repression of TGF- β signaling (through the expression of inhibitory SMAD7) (Azzolin et al., 2014; Qin et al., 2018; Xu et al., 2014), that are both involved in the myofibroblast gene program. In addition, this dimer can synergistically together with SRF/MRTF-A bind to promoters in cancer-associated fibroblasts (Foster et al., 2017), as well as in pro-fibrotic processes (Bialik et al., 2019). Recently, the role of crosstalk between SRF/MRTF-A and Yap/Taz has been specifically described as a critical modulator of α -SMA expression (Figure 3) (Speight et al., 2016). In this specific model, there is reciprocal inhibition between MRTF-A and Taz, leading to antagonistic effects on α -SMA expression that are context-dependent but mechanosensitive. It remains unclear whether such an interplay between MRTF-A and TAZ does exist in cardiac fibroblasts, and perhaps even plays a role during aging (Table 3). However, this should represent an interesting subject of investigation for the future.

7.3 Sirtuins

In addition to acute transcriptional effects, the persistence of a triggered gene program requires longer-term modification of the chromatin opening frame shift (Lai and Pugh, 2017; Sartorelli and Puri, 2018; Stillman, 2018). In multiple models of fibrosis, it has been indeed demonstrated that fibroblasts accumulate histone modifications, such as methylation or acetylation (Chelladurai et al., 2019; O'Reilly, 2017). Sirtuins represent a metabolic-sensitive family of histone deacetylases that are specifically involved in myofibroblast maturation and fibrotic development (Cencioni et al., 2015; Ponnusamy et al., 2015; Wyman and Atamas, 2018). Among the different Sirtuin family members, Sirtuin-1 plays a preponderant role in α -SMA and ECM gene expression although through a chemical TGF-

β /SMAD3 pathway in fibroblasts (Zerr et al., 2016). The use of resveratrol (activator of Sirtuin 1) mitigates doxorubicin-induced fibrosis and diastolic dysfunction, notably by promoting the decrease in α -SMA and metalloproteinase 2, an ECM modifying enzyme (Cappetta et al., 2016). Interestingly, some of these dysfunctions are alleviated by the activation of the Sirtuin1/AMP kinase axis (Gu et al., 2018). Therefore, a feedback between ECM stiffness modulation, mechanosensing response and Sirtuins may exist, potentially contributing to the phenotype of the aging cardiac fibroblast.

Cell senescence is also associated with metabolism imbalance and oxidative stress generation (Gu et al., 2018), to which Sirtuins are particularly sensitive (Gambini et al., 2011). Therefore, there may be a continuous combination of crosstalk and feedback between both metabolic and mechanical aspects in the aging fibroblast that could be orchestrated through the Sirtuins.

In the nucleus, mechanosensing is translated into a transcriptional program that initiates the myofibroblast maturation. Here, we focused our attention on two main transcriptional axes that seem to be relevant to mechanosensing. In fact, the SRF/MRTF-A axis is actin regulated, and SRF expression and regulation is known to be affected in the aging heart, while Yap/Taz has been described as being sensitive to ECM stiffness (Figure 3). SRF/MRTF-A and Yap/Taz share promoter regions (Figure 3); therefore they may inhibit each other's binding. In addition, here we discussed the chemical impact of the metabolic-sensitive deacetylases Sirtuins, which are known to be particularly involved in aging. Sirtuins, and notably Sirtuin-1, can have an impact on α -SMA transcription and ECM composition via the TGF- β /SMAD pathway, but the role of Sirtuins remains unknown in the aging cardiac fibroblast and thus represents an interesting research topic for future investigations.

8. Impact of cardiac cell senescence on fibroblast mechanosensing

During cardiac aging, some alterations to the fibroblast phenotype are in response to changes in other cell types. For example, changes in the cardiomyocyte phenotype and function can result in functional and structural changes in the heart. Cardiomyocytes in the aging heart gradually become dysfunctional, and they die (Bernhard and Laufer, 2008; Sheydina et al., 2011). The decreased ratio of cardiomyocytes to non-cardiomyocytes in the aging heart (Anversa et al., 1990) results from changes in three different mechanisms: proliferation, apoptosis, and senescence. The low rate of postnatal cardiomyocyte proliferation is even lower in the aging heart, decreasing to 0.45% per year (Anversa et al., 1990). Resulting from stress, which comes from a life-long accumulation of damages, molecular changes in pathways governing survival are altered, resulting in increased cell death and reduced survival. Even though some components of the caspase cascade are decreased in aging, there is a consensus that cardiomyocyte death is increased in the aging heart (Goldspink et al., 2003). Cell death by necrosis induces inflammation (Rock and Kono, 2008) and leads to the generation of reactive oxygen species that particularly alter mitochondrial homeostasis, further leading to changes in energy generation and compromised function. To compensate for the cardiomyocyte loss and as a result of ongoing low-grade inflammation, fibroblasts increase their production and processing of ECM, which affects their mechanosensing

pathway. Eventually, due to the paracrine effects of neighboring cells and as a response to the altered environment, fibroblasts acquire a senescent phenotype. The senescence-associated secretory phenotype is characterized by reduced proliferation and enhanced secretion of cytokines, ECM components, and proteases that further contribute to cardiac remodeling and dysfunction (Xie et al., 2017).

9. Innovation

We have recently examined fibroblast activation in the aging heart (Trial and Cieslik, 2018), where we concentrated on the role of fibroblasts in the pathological processes such as reparative fibrosis (healing after myocardial infarction) and adverse interstitial fibrosis. We also covered various pathways leading to activation of fibroblasts such as canonical TGF- β -Smad, ERK1/2, angiotensin II, and adrenergic β 2 pathways and finally addressed the issue of fibroblast heterogeneity.

In this current review, we concentrated on the pathway leading to α -SMA activation, but instead of the TGF- β dependent pathway, here we described the differences in mechanosensing. Mechanosensing represents an essential component in response to ECM tension and is a critical effector of the fibrotic reaction in myofibroblasts. We have reviewed the effector mechanisms operative in this response and proposed the possible major modulators of fibrosis in the aged heart. We specifically detailed changes (that occur in the aging fibroblast or heart) that have an impact on FA, actin polymerization and transcription factor translocation to the nucleus that affect α -SMA transcriptional program. We caution that some of the studies cited include data from other biological models extrapolated to our model; our results represent a hypothesis that will serve in constructing future studies.

10. Conclusions

In the aging heart, the mechanosensing driving force is altered at critical checkpoints, which finally leads to an impaired myofibroblast maturation (Figure 4). Briefly, ECM composition undergoes an enrichment in collagen I, biglycan, decorin, glycoproteins and FNEDA variants and a decrease in elastin. These severe changes, resulting in a stiffer matrix, apply a drastically different mechanical tension to the cells (Figure 4). FA sites are at the frontline of ECM changes, and they are particularly sensitive to this tension shift. Hence, not surprisingly, downstream age-related modifications include the effectors of the FA core complex, as well as changes in integrin subunits, an increased protein level of vinculin, and a decrease in JNK activity (Figure 4). Regarding the complexity of the adhesome that coordinates FA assembling, dynamics and signaling, the role of the FA core certainly remains underestimated and may require more in-depth investigations in the future.

The cytoskeleton, and especially the actin treadmill, represents the true mechanical part of the mechanosensing axis, but it also works as an integrative platform for both downstream and upstream signaling effectors. The decrease in F/G actin ratio is a typical age-related feature that has been reported by other teams (Lai and Wong, 2020) and by us (Cieslik et al., 2011b), in both cardiac fibroblasts and other cell types (Figure 4). Furthermore, we also demonstrated that the decreased α -SMA expression is one of the main deleterious features

of impaired cardiac myofibroblast maturation, leading to weaker contractile abilities. Yet, these findings remain empirical, since little is known about the precise mechanism that promotes actin cytoskeleton disorganization. A link between FA signaling and actin can be strongly suspected, but it only remains extrapolated from the literature in other cell types or acute models. In this sense, there are also indirect clues about the impact of fibroblast aging on actin itself, in terms of post-translational modifications. The enzymatic-redox balance that has been recently described as a part of the actin dynamic treadmill could be a part of the aging-affected machinery as well. But this remains hypothetical and may require extensive clarifications in cardiac fibroblasts.

Beyond its role in the cytoskeleton, actin also takes an active part in transcriptional machinery and notably in the SRF/MRTF-A axis that is known to trigger the myofibroblast gene program and especially α -SMA gene expression. Consequently, an altered actin treadmill may affect SRF/MRTF-A transcriptional activity directly and also RNA transcription, thus leading to the rapid shutdown of myofibroblast transition. Noteworthy, SRF binding capacity to the promoter of its target genes is indeed altered in the aging human and rodent heart (Lu et al., 1998; Takahashi et al., 1992; Zhang et al., 2011), while SRF protein level is increased (Lu et al., 1998), suggesting feedback adaptation (Figure 4). Nonetheless, observations from the last decades have not provided a direct link between fibroblast aging and SRF activity. In addition, a stiffer matrix is also known to impact another crucial transcriptional axis; the Yap/Taz and Hippo pathways, that have been extensively studied in cardiomyocyte regeneration and reprogramming (Liu et al., 2015a). Yet, because this change in matrix composition after injury in young hearts resembles ECM modification in the aging heart (Figure 4), we could logically suspect an indirect impact of ECM on Yap/Taz transcriptional axis, which remains mainly unexplored. In addition, an interesting crosstalk/competition relationship has been recently described between Taz and MRTF-A (Speight et al., 2016). However, there is still a missing experimental link in the case of aging cardiac fibroblasts.

Last but not least, recent transcriptional data suggest heterogeneity in aging fibroblast populations (Ali et al., 2014; Farbehi et al., 2019; Mahmoudi et al., 2019; Vidal et al., 2019), in such a way that there might be not only a unique defective signature but a heterogeneous population of aged and young-like cells, which makes the whole complexity challenging to address by either in vitro or in vivo studies (Figure 4).

Finally, a better understanding of mechanosensing (in the specific case of cardiac fibroblasts in the aging heart) remains an important way to address and explore these recurrent questions about myocardial integrity and dysfunction in the elder heart.

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Highlights

- Mechanosensing is the ability of a cell to respond to mechanical signals such as force or rigidity
- Fibroblasts transition into contractile myofibroblasts upon chemical and mechanical signals
- Matrix, integrins, focal adhesions and cytoskeleton play essential parts in mechanosensing
- In the aging heart, myofibroblast transition is impaired leading to dysfunction after infarct

Figure 1.
Schematic representation of fibroblast-myofibroblast maturation in the young and old heart.

Figure 2: Schematic representation of the Focal Adhesion (FA) core complex.

The anchorage of integrin subunits to the ECM promotes the recruitment of talin (Tal), paxillin (Pax), kindlin-2 (K), which facilitates the docking and catalytic activity of the integrin-linked kinase (ILK) and focal adhesion kinase (FAK). FAK ensures the recruitment and the binding of Src, and the enrichment of the FA with phosphoinositide (PIP2). Downstream signaling of FAK, integrin-linked kinase, and Src includes RhoGTPase that can trigger actin polymerization to generate fibers (F-actin) or MAP Kinase pathways. Enzymatic activity at the FA core also favors the recruitment of actin-binding proteins such as: vinculin that binds to Pax and reinforces the tight connection between the FA complex

and the cytoskeleton, α -actinin that helps the stabilization of parallel F-actin, Arp2.3 (Arp) or N-WASP that favor branching of F-actin.

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Figure 3: Signaling in the nucleus that regulates the myofibroblast gene program.

The mechanosensing differs between an elastic (left) or a stiffer (right) ECM, leading to downstream impacts on MRTF-A and Yap/Taz shuttling dynamics. The DNA binding sites of SRF and TEAD transcription factors (CARG and MCAT boxes, respectively) can overlap within the promoter region. So, both MRTF-A and Yap/Taz share common target genes, suggesting crosstalk or competitions. This crosstalk seems to be notably involved in the α -SMA expression.

Figure 4: Alterations of mechanosensing in fibroblasts from aged hearts.

An altered extracellular matrix (ECM) composition is causally related to a changed phenotype in fibroblasts, leading to impairment at the FA, affecting the downstream cytoskeleton treadmill and myofibroblast gene program, which worsens the negative feedback of the mechanosensing. Evidence concerning the role of mechanosensing in aging fibroblasts has been accumulated both in the myocardium (pink squares) or other tissues (yellow squares).

Table 1**Abbreviations.**

Abbreviation	Full name
ART	Ak strain Transforming, Protein Kinase B
Arp2/3	Actin Related Proteins 2/3
Cdc42	Cell Division Control Protein 42 homolog
ECM	Extracellular Matrix
F-actin	Filamentous Actin
FA	Focal Adhesions
FAK	Focal Adhesion Kinase
FERM	Protein 4.1-Ezrin-Radixin-Moesin
FN	Fibronectin
FN-EDA	Fibronectin with Extra Domain A
G-actin	Monomeric Actin
GSK3	Glycogen Synthase Kinase-3
GTPases	Guanosine Triphosphatases
JNK	c-Jun N-terminal Kinase
LATS1/2	Large Tumor Suppressor Kinase 1/2
MAP kinase	Mitogen Activated Protein Kinase
MDia	Mammalian Diaphanous
MRTF-A	Myocardin-Related Transcription Factor A
MST1/2	Mammalian Sterile 20-like Kinases 1/2
mTOR	Mammalian Target of Rapamycin
NFAT4	Nuclear Factor of Activated T-cells 4
N-WASP	Neural Wiskott-Aldrich Syndrome Protein
PDGFR α	Platelet Derived Growth Factor Receptor α
PI3K	Phosphatidylinositol-3-kinase
Rac1	Ras-related C3 botulinum toxin 1
Rho GTPase	Ras homolog GTPase
SMAD 3, 4, 7	(Small) Mothers against decapentaplegic homolog 3, 4, 7
α -SMA	α -Smooth Muscle Actin
Sos	Son of sevenless
SRF	Serum Response Factor
Taz	Transcriptional co-Activator with a PDZ motif
TGF- β	Transforming Growth Factor- β
TRP	Transient Receptor Potential
TRPV4	Transient Receptor Potential Vanilloid 4
Yap	Yes-Associated Protein
ZBP-1	Zip-code-Binding Protein 1

Table 2

Actin Dynamics in Mechanosensing Downstream from the Focal Adhesion.

The role of actin in mechanotransduction		
	Normal role in fibroblast physiology	Age-related changes
<i>Actin as part of the cytoskeleton</i>	In mechanosensing, the actin cytoskeleton rearranges in response to ECM tension (Grabec et al., 2000) and takes part in fibroblast-myofibroblast differentiation (Omelchenko et al., 2002).	Aging is associated with drastic biochemical changes that may alter actin polymerization and expression (Cieslik et al., 2011; Lai and Wong, 2020). Oxidative stress can alter actin turnover and/or filament organization, due to ROS or methionine sulfoxidation imbalance (Aberle, 2013; Varland et al., 2019). The impact of oxidative stress in actin polymerization has been demonstrated in eukaryotic cells (Farah et al., 2011) and adult cardiomyocytes (Angelini et al., 2020) but has not been shown in cardiac fibroblasts.
<i>Actin in fibroblast maturation</i>	During their maturation, myofibroblasts express α -SMA, a contractile actin isoform, in their cytoskeleton (Hinz et al., 2001, 2003); this favors wound healing (Hinz, 2006; Santiago et al., 2010).	Aged cardiac fibroblasts show decreased α -SMA isoform, along with a decreased and disorganized F-actin network leading to impaired cardiac fibroblast contractility (Cieslik et al., 2011).
<i>Actin and mRNA "zip-coding"</i>	The fibroblast is the only cell type in which mRNA zip-coding (cytoplasmic sorting of mRNA) persists after embryogenesis (Jansen, 2001; Lopez de Heredia and Jansen, 2004). β -actin mRNA is reported to be extensively zip-coded and enriched at the FA site (Bassell et al., 1998). Integrin signaling promotes ZBP phosphorylation, and therefore mRNA zipcoding in fibroblasts (Bassell et al., 1998; Chicurel et al., 1998; Kislauskis et al., 1994; Shestakova et al., 2001). F-actin binds ZBP-1 and ensures the zip-coded mRNAs localization through polymerization (Nicastro et al., 2017).	Modulation of mRNA turnover by age-related factors (Borboldis and Syntichaki, 2015) could potentially impair mRNA zip-coding. Its role remains unknown in the aging heart.

Table 3**Mechanosensing and Gene Regulation in the Maturation of Cardiac Fibroblasts.**

Transcription factors in the regulation of α-SMA expression		
	Normal role in fibroblast physiology	Age-related changes
<i>SRF/MRTF-A</i>	<p>SRF/MRTF-A is involved in the myofibroblast maturation gene program (Esnault et al., 2014; Small, 2012) and in α-SMA expression (Medjkane et al., 2009; Olson and Nordheim, 2010; Selvaraj and Prywes, 2004).</p> <p>MRTF-A is actin-regulated: G-actin promotes its cytosolic retention (Miralles et al., 2003; Percipalle et al., 2003; Posern et al., 2004, 2002).</p> <p>F-actin formation from mechanical/biochemical stimuli promotes MRTF-A translocation to the nucleus and dimerization with SRF (Miralles et al., 2003; Posern et al., 2004, 2002).</p> <p>Its role is documented in cardiomyopathy models (Chang et al., 2003; Davis et al., 2002) as well as in fibrosis and scar formation (Gary-Bobo et al., 2005; Parlakian et al., 2005).</p>	<p>A two-fold increase in SRF level in the heart has been seen in aging (Lu et al., 1998; Takahashi et al., 1992). The upregulation of SRF remains unknown in aging cardiac fibroblasts.</p> <p>SRF promoter-binding capacity is impaired with aging and pressure overload (Lu et al., 1998; Zhang et al., 2011).</p> <p>Competition with other transcription factors or diminished co-factor availability may alter the final gene transcript (Esnault et al., 2014; Lin et al., 2007, 2008; Prywes and Zhu, 1992; Sun et al., 2009).</p> <p>Age-related actin depolymerization (Farah et al., 2011; Varland et al., 2019) may lead to MRTF-A cytosolic retention.</p>
<i>Yap/Taz</i>	<p>Yap/Taz integrates the FA signaling as part of the mechanosensing response (Ma et al., 2019; Piccolo et al., 2014).</p> <p>Yap/Taz is sensitive to ECM tension via the Hippo pathway; an elastic matrix favors Yap/Taz degradation (Ma et al., 2016).</p> <p>Yap/Taz activates myofibroblast differentiation in a model of dilated cardiomyopathy (Jin et al., 2018).</p> <p>Yap/Taz leads to repression of TGF-β signaling via beta-catenin degradation affecting myofibroblast gene program (Azzolin et al., 2014; Qin et al., 2018; Xu et al., 2014).</p> <p>Yap/Taz antagonizes the SRF/MRTF-A transcriptional axis on α-SMA expression (Speight et al., 2016).</p>	<p>The role of Yap/Taz remains unknown in the aging cardiac fibroblasts.</p> <p>Stiff ECM, as seen in the aging heart, promotes Yap/Taz nuclear translocation and competition with SRF/MRTF-A (Liu et al., 2015).</p>