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Cellular cross-talks in the diseased and aging heart

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A B S T R A C T

Communication between cells is an important, evolutionarily conserved mechanism which enables the coordinated function of multicellular organisms. Heterogeneity within cell populations drive a remarkable network of cellular cross-talk that allows the heart to function as an integrated unit with distinct tasks allocated to sub-specialized cells. During diseases and aging, cells acquire an overt disordered state that significantly contributes to an altered cellular cross-talk and hence drive cardiac remodeling processes and cardiovascular diseases. However, adaptive mechanisms, and phenotypic changes in subpopulations of cells (e.g. reparative macrophages or fibroblasts) can also contribute to repair and regeneration. In this article, we review the cellular cross-talks between immune cells, endothelial cells, fibroblasts and cardiomyocytes that control heart failure by contributing to cardiac dysfunction and aging, or by mediating repair and regeneration of the heart after injury.

Disorders or diseases of the cardiovascular system are still the primary cause of death globally [1,2]. Although the heart and the cardiovascular system has been studied for centuries, researchers mainly focused on cardiomyocyte functions and cardiac fibrosis as key mechanisms of heart failure. However, the more recent reports showing that cardiomyocytes only account for up to 35% of the total cells of the heart [3–5] inspired a more integrated view of cardiac biology. Many cell types including endothelial cells, pericytes, smooth muscle cells, various immune cells, fibroblasts/mesenchymal cells and nerves contribute to the cellular composition of the heart. This diverse set of cells together maintains cardiac function and is the key to control repair and cardiac regeneration. In the present review, we focus on the interplay of immune cells and the three cell populations namely cardiomyocytes (CM), fibroblasts (FB) and endothelial cells (EC) during disease, regeneration and aging. The heterocellular cross-talk in the heart under physiological conditions was recently reviewed by Perbellini et al. [6]. The first two chapters will focus on detrimental and beneficial changes in cellular communication as it occurs during cardiac disease and regeneration, while the last chapter will discuss cellular changes during cardiac aging.

1. Cellular cross-talks in the diseased heart

In the past, cellular cross-talks within hearts have been studied in various animal and disease models including acute myocardial infarction (MI), trans-aortic constriction (TAC), heart failure with preserved ejection fraction and diastolic dysfunction and during aging. During MI, the occlusion of a coronary artery results in a reduced blood and nutrient supply in the underlying tissue causing cell death. This leads to a dramatic change in the cellular composition within the heart tissue: reduced numbers of CMs, ECs and FBs are replaced by various immune cells within the first days upon MI [7] (Fig. 1A). The initial inflammation driven phase is followed by increased EC and FB proliferation, whereas CMs - due to their limited proliferation capacity- remain depleted in the scar tissue of adult mammalians. Interestingly, the loss of cells after injury can be counterbalanced in zebrafish [8,9] or newts [10] as well as in neonatal mice [11,12], which are able to regenerate the myocardium after injury. However, unlike the zebrafish heart whose regenerative capacity is preserved throughout the entire lifespan [13], the regenerative capacity of the murine heart is decreasing after postnatal day 7 [11,12,14,15]. In contrast to MI, the changes of cell compositions upon TAC are less dramatic but also involve invasion of inflammatory cells and an adaptive response of CM-EC communication to cope with the increased oxygen demands of the hypertrophic CM. The interactions occurring in models of HFpEF and aging are less well studied but certainly involves an increase in fibrosis and extracellular matrix remodeling, which affects other cell types of the heart.

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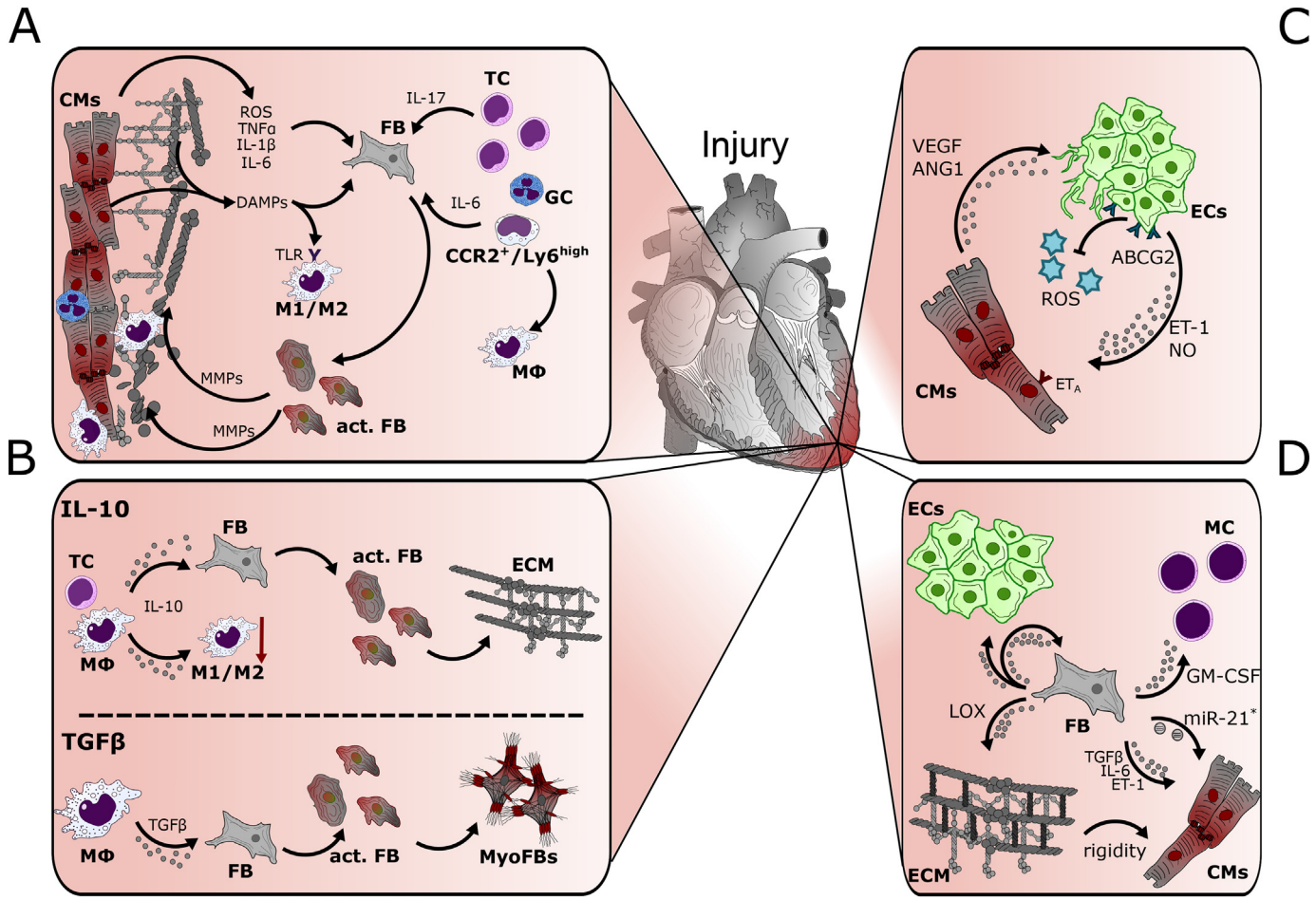

Fig. 1. Cellular cross-talk in the injured heart. (**A**) Cardiac injury and lack of oxygen stimulates cardiomyocytes (CMs) in the injured heart to release pro-inflammatory factors (ROS, TNFα, IL-6 and IL-1β) into the extracellular space. T cells (TC), granulocytes (GC) and CCR2^{+}/Ly6^{high} monocytes infiltrate the injured area and release further pro-inflammatory factors (IL-6, IL-17). The released factors stimulate fibroblasts (FBs) to become activated FBs (act. FBs). Damaged extracellular matrix (ECM) components and hypoxic CMs additionally release danger-associated molecular patterns (DAMPs) that further activate FBs and also stimulate M1 and M2 macrophages (MΦ) via toll-like receptors (TLR). Activated FBs secrete matrix metalloproteases (MMPs) that degrade the ECM and enables the infiltration of phagocytes to remove cell and matrix debris. (**B**) TC- and MΦ-derived IL-10 reduces the ratio of pro-inflammatory M1 to M2 macrophages thereby eliciting an anti-inflammatory effect. IL-10 further activate FBs to produce ECM components in order to form the scar tissue in infarcted hearts. (**C**) Hypoxic CMs release pro-angiogenic factors (VEGF, ANG1) to induce revascularization, whereas endothelial cells (ECs) interacts with CMs by releasing ET-1 and nitric oxide (NO). To neutralize ROS concentration, ECs transport gluthathion (e.g. via the ATP-binding cassette transporter ABCG2). (**D**) FBs cross-talk with a variety of cardiac cells. By secreting granulocyte–macrophage colony-stimulating factor (GM-CSF), FBs recruit monocytes, but also signal to ECs via paracrine and autocrine factors. CM hypertrophy is induced by FB-derived TGFβ, IL-6 and ET-1 as well as exosomes loaded with miR-21*.

1.1. Inflammatory cells in heart disease

Immune cells represent a minor population in the heart under steady state conditions, although all major leukocyte classes are represented including monocyte/macrophages, neutrophils, T- and B-cells and dendritic cells [16]. However, invasion of bone marrow-derived inflammatory cells is a hallmark in most if not all cardiac disease models. MI leads to a rapid accumulation of mast cells and neutrophils followed by an activation of tissue resident macrophages and invasion of inflammatory C–C chemokine receptor 2 positive (CCR2^{+}), Ly6^{high} monocytes [17,18] (Fig. 1A). Especially tissue resident TIMPD4^{+} macrophages were found to proliferate in the peri-infarct zone and reinforce the macrophage pool in the injured heart [19]. In models of pressure overload and HFpEF, CCR2^{+} macrophages also infiltrate the heart [20,21]. For a detailed description of the various immune cell subsets in cardiac disease, the reader is referred to Swirski & Nahrendorf [22].

The inflammatory response has a double-edged role in the regulation of remodeling and heart failure [23]: Whereas early stimulation of inflammatory signaling is important for clearance of dead cells from the infarcted tissue, excessive inflammatory injury contributes not only to pathophysiological remodeling of the ventricle but also induces reparative processes. These processes are mediated by a variety of cytokines which interact with non-leukocytes. These include for example mast cell-derived tumor necrosis factor (TNF), which activates endothelial cell [24], and T cell-derived IL-17, which stimulates cardiac fibroblasts [25]. Macrophage and T-cell derived factors can also induce reparative processes (Fig. 1B). E.g. transforming growth factor- β (TGFβ) and IL-10 [26,27] promote collagen production and resolution of inflammation, whereas vascular endothelial growth factor (VEGF) [28] promotes neoangiogenesis (for details see [22]). **TGFβ**, however, can also have detrimental effects [29]: It promotes cardiomyocyte death and hypertrophy and mediates cellular differentiations processes, such as fibroblast-to-myofibroblast and endothelial-to-mesenchymal transition (EndoMT) [30,31], which both increase the pool of active mesenchymal cells and may contribute to scar formation and detrimental cardiac remodeling [31].

Endothelial-to-mesenchymal transition (EndoMT) is a complex process by which ECs adopt mesenchymal features such as cellular motility and contractile element. ECs that undergo EndoMT lose the

expression of endothelial-specific genes including vascular-endothelial cadherin and CD31 but acquire mesenchymal transcriptomic signatures by expressing –among others– SM22α, S100A4, fibronectin, N-cadherin, type I and III collagen [32]. The process is induced by many different stimuli. In the injured and diseased heart, mostly inflammatory or pro-fibrotic factors such as TGFβ and hypoxia drive EndoMT [33]. During cardiac development EndoMT plays a critical role. While it has been shown that about 10% of ECs in human fetal cardiac valves undergo EndoMT, only 1% remain in adult valves [34]. However, in adulthood EndoMT can also be maladaptive and displays a role in valvular diseases [35]. Furthermore, EndoMT was shown to occur during MI [36], in atherosclerotic lesions and was associated with plaque erosions [37]. For a detailed description of EndoMT in development and disease, the reader is referred elsewhere [32,35].

IL-10 reduces the ratio of pro-inflammatory M1 to M2 macrophages in MI models thereby eliciting an anti-inflammatory effect [38,39] (Fig. 1B). Consistently, IL-10-deficient mice have increased infarct size and enhanced myocardial necrosis with increased neutrophil infiltration [40]. Although its role as anti-inflammatory mediator, which improves cardiac function after injury in the MI model is well established, a recent study suggest that it may have mild, but adverse effects if released by macrophages in a model of diastolic dysfunction [21]. A common effect observed in diastolic dysfunction [21] and post-infarction remodeling [39] is that IL-10 activates FBs. Since FBs play an important role in wound healing and cardiac remodeling their activation may contribute to the beneficial effect of IL-10 in acute injury models, whereas a long term chronic activation of FBs may be detrimental to the heart. In this regard, Hulsmans et al. [21] examined cardiac macrophages in two different approaches to study diastolic dysfunction by using first a combination of salty drinking water, unilateral nephrectomy as well as chronic exposure to aldosterone and second a mouse model of physiological aging. They documented that the density of myocardial macrophages and IL-10 expression increased upon diastolic dysfunction, whereas IL-10 expression shifted macrophages in an autocrine manner to a more pro-fibrotic subset, and additionally activated FBs resulting in increased FB proliferation and elevated collagen deposition and thereby to ventricular wall stiffness. By contrast, a macrophage-specific IL-10 depletion improved cardiac filling during diastole [21].

1.2. Endothelial cross-talks

Cardiac ECs form the inner surface of the heart chambers, as well as the inner lining of the entire macro- and microvasculature supplying the myocardium with blood. To fulfill the high oxygen demands, a capillary ratio of > 1 capillary/myocyte is required and hence it is not surprising that ECs outnumber CMs by a ratio of 3:1 [41]. However, the total EC content has not been uniformly defined yet since it varies depending on the methodologies that were used. For example, Pinto et al. reported by immune fluorescence staining that ECs make up 60% of the non-myocytes in the murine heart [16], while using histological techniques, Perbellini et al. described that ECs account for 48% of the entire heart [42]. On the other extreme, Banerjee and co-workers showed via FACS studies that the EC content of the entire mouse heart is only 7% [43]. Admittedly, one has to keep in mind, that tissue dissection can be very harsh and might digest even membrane surface proteins such as CD144 or CD31 on ECs preventing capturing these cells in flow cytometry studies. However, various studies have consistently reported changes in EC numbers and functions within the heart upon injury. After an initial decline in EC after infarction which is caused by cell death, new blood vessels growth between 7 and 14 days after injury. In models of pressure overload, adaptive angiogenesis and neovascularization is augmented to fulfill the increased oxygen demand of the hypertrophic CM [44]. A failure of angiogenesis in both models leads to the progression of HF [45].

In the heart, EC closely interact with CMs already during development, e.g. by releasing neuregulin, neurofibromatosis type-1 (NF1) and platelet-derived growth factor β (PDGFβ) [6,46–48]. After cardiac injury, a major function of ECs was attributed to the control of immune cell invasion [49] via up-regulation of P-selectin [50] as well as MCP-1 [51] and the restoration of the vascular network to provide oxygen to the ischemic or hypertrophic cardiac tissue. Several studies, however, suggest that EC have important functions beyond acting as inflammatory gate keeper or oxygen pipelines [52,53]. In other organs, such as the liver or lung, a paracrine activity of ECs (so called “angiocrine” mediators) was shown to control regeneration [54,55]. In the heart, EC can release many factors that control FB and CM functions but a direct pro-regeneration activity has not yet been reported.

Important insights into CM-EC communication paths were provided by studies showing that hypertrophic CM secrete pro-angiogenic factors such as VEGF or angiopoietin-1 to promote adaptive vessel growth. Inhibition of VEGF prevented increased capillary density and induced heart failure [44,56]. But EC can also signal back to CM: For example, EC derived endothelin-1 acts in a paracrine manner on CMs (via the ET_A receptor) and induces hypertrophy as well as remodeling in the diseased heart [6]. Other EC-derived factors were shown to elicit protective effects on CM. For example, apelin, which is mainly expressed by ECs, protects against the progression of heart failure in mice [57]. Likewise, nitric oxide, which is mainly produced by the endothelial nitric oxide synthase under baseline conditions, is known to inhibit CM hypertrophy [58]. However, disease or cardiac stress-related uncoupling of eNOS can promote oxidative stress and result in pathological cardiac remodeling [59]. EC also express transporters of gluthathione (such as the ATP-binding cassette transporter ABCG2), which enriches the extracellular space with this important antioxidant and reduces reactive oxygen species that mediate CM damage [60] (Fig. 1C).

Since the interpretation of in vivo studies targeting ECs are often hampered by potential confounding effects of increased or diminished perfusion, co-culture studies have been used to gain insights into direct EC-CM interactions. Such a study demonstrates that EC promote CM survival by reducing apoptosis and necrosis and promoted spatial remodeling as well as synchronized contraction of CMs by augmenting connexin 43 expression [61]. A cardiac protective role of ECs was further described by Kuramochi et al., showing that EC-derived neuregulin reduces apoptosis of CMs in a co-culture system [62].

1.3. Cardiomyocytes

CMs are the contractile element of the myocardium. Their synchronized contraction maintains a proper cardiac strain that is needed to pump blood through the circulatory system. To maintain cardiac contraction throughout a whole lifespan, CMs are equipped with a very large cytoplasm that is crammed with sarcomeres, the contractile unit of myocytes and mitochondria to meet their high energy demand [6]. The main interaction partners of CMs are neighboring CMs. The connection is established by specific gap junctions, so-called intercalated disks that are usually formed by connexins [6]. These junction proteins allow the direct exchange of ions, small molecules as well as small peptides between CMs and also electrically couple the entire myocardium [6].

CM also communicate with other cells, via secretion of various chemokines. For instance, CM-derived VEGF-A plays a crucial role during cardiac development by controlling the formation of capillaries [63]. In addition to the bidirectional interplay of CM with EC and inflammatory cells described above, CM death itself can contribute to the pro-inflammatory extracellular milieu. Thus, CM death or stimulation of CMs by immune cells cause the secretion of pro-inflammatory chemokines and cytokines, which further aggravates inflammation and activates neighboring EC or FB. For example, CM-derived IL-6, TNFα and IL-1β activates neutrophils and augment adhesion as well as invasion into the damaged tissue via expression of intercellular adhesion

molecule 1 [64]. Dying CMs and degraded ECM both further release danger-associated molecular patterns that promote additional inflammatory reactions via toll-like receptor (TLR) signaling [65]. Reactive oxygen species that additionally trigger the immune response, are increasingly formed and released by CMs upon hypoxic conditions [64]. However, deciphering the role of the innate immune system in myocardial diseases turned out to be challenging, because of conflicting observations that were made in ischemic heart diseases indicating both a beneficial and a detrimental role of cardiomyocyte TLRs upon cardiac injury. Whereas a short-term activation of TLRs was found to have a cytoprotective effect within the heart, long-term activation of TLRs is maladaptive resulting in increased pro-inflammatory cytokine release and adhesion molecule expression leading to the recruitment of neutrophils, dendritic cells and monocytes into the myocardium and adverse cardiac remodeling [66,67]. In the murine heart, at least six TLRs are expressed including TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 [68], of which TLR2 and TLR4 are best studied [69–71]. Interestingly, both TLR2 and TLR4 seem to have an anti-apoptotic effect in CMs upon serum-deprivation and LPS-stimulation [72,73]. TLR4 further mediates iNOS induction in CMs leading to an increased NO production that was documented to inhibit caspase-3 activity [74,75]. Frantz et al. demonstrated that the blocking of TLR2 in neonatal rat CMs oxidative stress-induced apoptosis and cell injury [76]. However, as mentioned above, TLR signaling may also be detrimental to heart including adverse cardiac remodeling. For instance, studies reported a reduced infarct size, neutrophil recruitment and ROS as well as cytokine release and augmented contractile performance and recovery in TLR2-deficient mice [77,78]. Similar findings were shown after deletion of TLR4 [79,80].

1.4. Fibroblasts

Cardiac FBs are small elongated spindle-shaped cells with a strong secretory phenotype. They display a granular cytosol containing a pronounced rough endoplasmic reticulum. Unlike CMs, that are organized in a defined structure within the heart, FBs are roughly distributed throughout the entire myocardium and are located in the interstitial space between CMs [81]. Studies on fibroblasts have been hampered by the lack of specific marker, since many of the proteins which are highly expressed on FBs such as vimentin or fibroblast-specific protein 1 (FSP1) are also expressed on other cells [82]. More recent studies used platelet-derived growth factor receptor α (PDGFRα), TCF21 or DDR2, which are considered to be more specific for FBs [82]. FBs derive from multiple origins including the epicardium, the endocardium and neuronal crest derived cells, which all give rise to FB during development. The origin of FBs that is responsible for cardiac fibrosis in the diseased adult heart has been controversial but most recent lineage tracing studies suggest a predominant contribution of tissue resident FBs [82]. FBs may directly interact with other cells via connexins possibly allowing an electric coupling with CMs [83].

Cardiac injury and subsequent inflammation induces dramatic changes in FB resulting in increased proliferation, differentiation and matrix production [84]. FBs thereby respond to many factors (e.g. IL-6, IL-10) provided by inflammatory cells, EC and CM but also can signal back to these cells. For example, fibroblast-derived granulocyte–macrophage colony-stimulating factor (GM-CSF) was shown to induce the production and recruitment of myeloid cells [85]. Moreover, bioinformatic assessment of single cell sequencing data sets suggest that FB are cells with a high expression of “outgoing” signals representing extracellular factors that can putatively interact with receptors expressed on other cardiac cells [7] (Fig. 1D). Such a view of FBs being major determinants of the microenvironment is consistent with findings in other tissues or pathologies demonstrating that FBs drive the microenvironment e.g. in cancer [86].

FBs are major producers of extracellular matrix components and modifying enzymes e.g. Lysl oxidases or proteases. Since CMs are physically linked to the ECM via integrin molecules [87], regulation of the ECM by FBs indirectly influences CMs. Of the numerous additional factors released by FBs (but also other cells), TGFβ, IL-6, and endothelin 1 have significant effects on CMs. FBs also secrete miRNA-loaded exosomes; here miR-21* loaded exosomes were shown to induce CM hypertrophy [88]. Recent studies also suggest that a FB subset can produce Wnt inhibitory factors, which may have autocrine or paracrine functions [7]. Interestingly, this FB subset was shown to be reduced after MI [7]. While these examples highlight the interaction of FBs with CM, bioinformatics analysis of single cell sequencing data sets suggest that the main interacting cell type of fibroblasts in the heart may be endothelial cells, which may trigger further research to elucidate the interactions between these two cell types [7,89].

2. Cellular cross-talks in regeneration

In contrast to model organisms such as zebrafish or newts, the adult mammalian heart has a limited capacity for regeneration. Recent studies, however, demonstrated that mice hearts can regenerate during the first week after birth [11,12,14,15]. The regenerative capacity has been mainly attributed to an increase in CM proliferation, which is lost during adulthood [14,15]. However, several studies are providing evidence that other cell populations are required as well to allow a successful regeneration of the cardiac tissue.

2.1. Inflammatory cells during cardiac regeneration

Pro-angiogenic macrophages can play an active role in cardiac regeneration by promoting vessel growth [90]. Macrophage subpopulations are also required for regeneration of hearts in in salamander [91]. In contrast to the transient extracellular matrix that normally accompanies regeneration in salamander hearts, macrophage depletion resulted in a permanent, highly cross-linked extracellular matrix scar derived from alternative fibroblast activation and lysyl-oxidase enzymes [91]. Interestingly, cardiac resident macrophage-derived cytokines can promote CM proliferation in hypoxic neonates [92] suggesting an additional potential role of macrophages in CM replacement. However, in salamander hearts, macrophage depletion did not interfere with CM proliferation [91] indicating that CM replacement may be controlled by a macrophage-independent mechanism in this setting (Fig. 2A). In zebrafish models, Bevan et al. [93] recently demonstrated that both *tnfα*$^{+}$ and *tnfα*$^{-}$ macrophage subsets manipulate cardiac regeneration. While *tnfα*$^{+}$ macrophages facilitate a pro-inflammatory response and promote scar formation via Csf1ra upon cryoinjury, *tnfα*$^{-}$ macrophages contribute to scar removal and resolution of the inflammatory response during regeneration [93]. For a more detailed description of the regenerative capacity of macrophages, the reader is referred to further review articles [94,95].

The important role of immune cells is further underlined by findings showing that T-cells are important players in regeneration [96–98]. Thus, zebrafish Treg-like (Treg) cells modulated inflammation and stimulated regeneration in different organs including the heart [99]. Interestingly, the pro-regenerative activity was mediated through interleukin-10-independent secretion of organ-specific regenerative factors, namely Neuregulin 1, which rescued the regeneration defects associated with Treg cell depletion [99]. In addition, Treg conditioned medium or overexpression of Treg secreted factors (Cst7, Tnfsf11, Il33, Fgl2, Matn2, and Igf2) reduced infarct size and augmented CM proliferation in infarcted mouse hearts [100] (Fig. 2B).

2.2. Fibroblasts during cardiac regeneration

Increasing evidence suggests that also specific FB populations may support CM growth and maturation through the secretion of specific ECM components. Therefore, it is not surprising that regenerated cardiac tissue in zebrafish and in neonatal mice is characterized by a

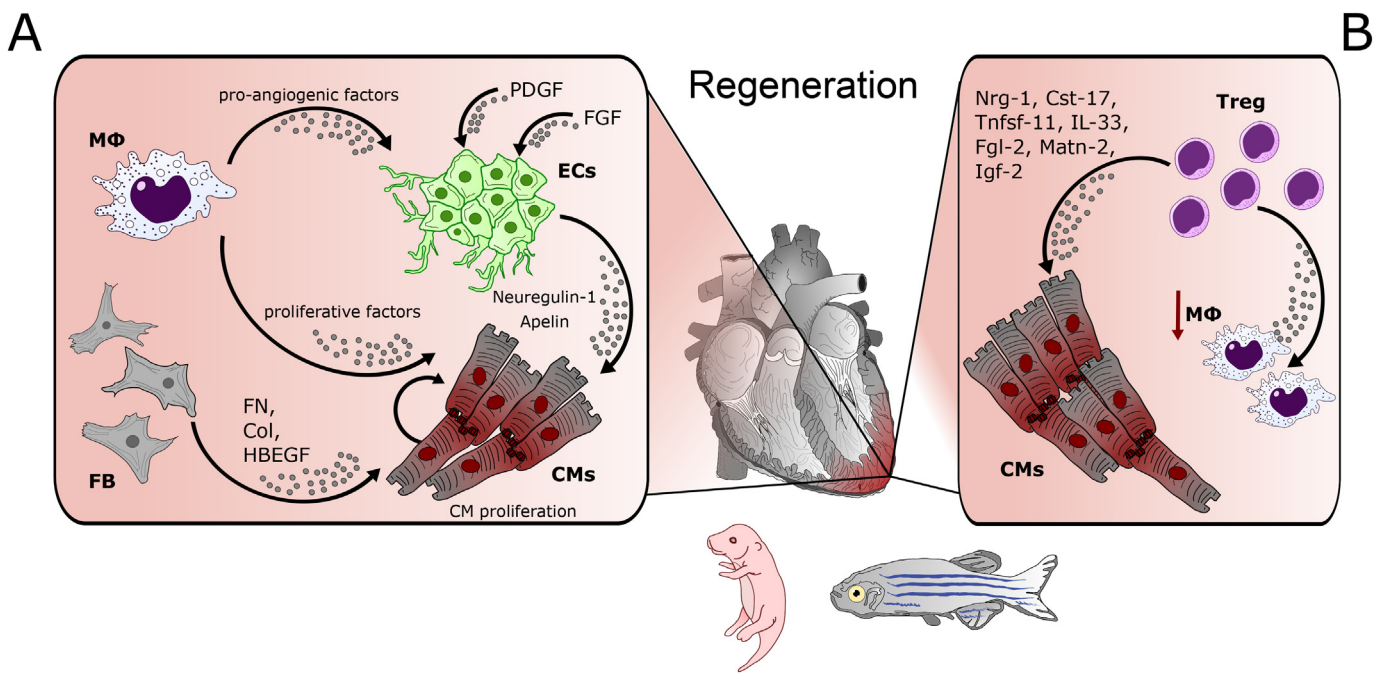

Fig. 2. Cellular cross-talk during regeneration. In contrast to adult mammalian heart, newts and zebrafishes are capable to regenerate their hearts. (**A**) Macrophage- (MΦ-) derived pro-angiogenic factors as well as FGF and PDGF stimulate endothelial cell (EC) sprouting to induce revascularization. Cardiomyocyte (CM) proliferation is driven by fibroblast- (FB-) derived FN, Col and HBEGF, EC-derived neuregulin-1 and apelin as well as MΦ-derived proliferative factors. (**B**) Regulatory T cells (Treg) further induce CM proliferation by releasing Nrg-1, Cst7, Tnfsf11, Il33, Fgl2, Matn2, and Igf2 and attenuate MΦ activation.

fibroblast-rich scar tissue [82]. In line with this, embryonic cardiac FB were shown to induce CM proliferation in vitro, whereas adult cardiac FB rather promoted myocyte hypertrophy [101]. Fibronectin, collagen and heparin-binding EGF-like growth factor were identified as embryonic cardiac fibroblast-specific signals that collaboratively promoted via integrin beta 1 CM proliferation in a paracrine fashion [101]. The responsible FB (sub) population that promotes regeneration in adult hearts remains to be identified. They may be remnants of embryonic FBs, epicardial-derived cells or other pro-regenerative fibroblast population described in the adult heart [81] (Fig. 2A).

2.3. Endothelial cells during cardiac regeneration

A rapid restoration of oxygen supply by growth of new capillaries and vessels is key to regeneration. Besides re-establishing perfusion, ECs express a number of cardio-affective paracrine factors to restore CM survival and function. Bassat et al. recently reported that ECs express the extracellular matrix protein agrin, which enhances CM proliferation in mouse- and human-derived iPS cells in vitro and improve cardiac repair in vivo upon MI [102]. ECs also synthesize nitric oxide (NO) by the endothelial NO-synthase, which has a protective role during cardiac damage [103]. Whether NO also affects cardiac regeneration, however, is unclear.

2.4. Cholinergic nerves during cardiac regeneration

Since 1950 we know that nerves play an important role during tissue regeneration: limb regeneration is impaired, if the intervening nerve is severed prior to or shorty after limb amputation [104]. These results were further confirmed in 1960s and the regenerative effect was linked to cholinergic nerves [105,106]. Interestingly, Mahmoud et al. transferred these regenerative effects to the cardiovascular field and showed that pharmacological inhibition of sympathetic nerves reduces CM proliferation in zebrafish and neonatal mice after MI [107,108]. The reduction in CM proliferation was rescued by using nerve growth factor and neuregulin-1 [108].

3. Changes in cellular cross-talks during aging

Aging is the major risk factor for decline in cardiovascular physiological functions and development of cardiovascular diseases. On macroscopic level, characteristic cardiac remodeling processes can be observed that mostly affect the cardiac chamber geometry, including age-related dilation of the left atrium accompanied by myocardial fibrosis [109]. Further structural alterations can be found in the left ventricle that undergoes cardiac hypertrophy, increased wall thickness and chamber dilation, as well as interstitial fibrosis [110]. Apart from chamber abnormalities, age-related valve diseases also occur with aortic valve stenosis being the most prevalent one [111]. Furthermore, diastolic function also declines with advanced age, whereas concomitant systolic dysfunction was not reported [112]. However, Feridooni et al. noted that the ability to augment ventricular function is disturbed under high demand situation, such as exercise, in old adults, while the systolic function at rest is not affected [112]. Cellular and molecular processes that underlie these cardiac alterations can be quite diverse. Replicative senescence caused by shorting of telomeres [113–115], increased oxidative stress [116] and DNA damage [117], deregulation of genes and proteins, impaired cell-cell communication, and an altered systemic and local environment cause the eventual demise of cells [118]. Many of these changes are described in the aging heart. In cardiomyocytes, however, telomere shortening likely is caused by telomere erosion [119].

3.1. Senescent cells and SASP in the aging heart

Cellular senescence is characterized by a growth arrest and is part of a physiological process that maintains tissue homeostasis and limits tumor progression. However, it has also been implicated as a major cause of age-related disease in part mainly because senescent cells release a pro-inflammatory senescent associated secretory phenotype (SASP) [120] (Fig. 3A). The secretome of senescent cells include soluble signaling molecules, secreted proteases as well as insoluble/extracellular matrix components. Examples are inflammatory cytokines (e.g.

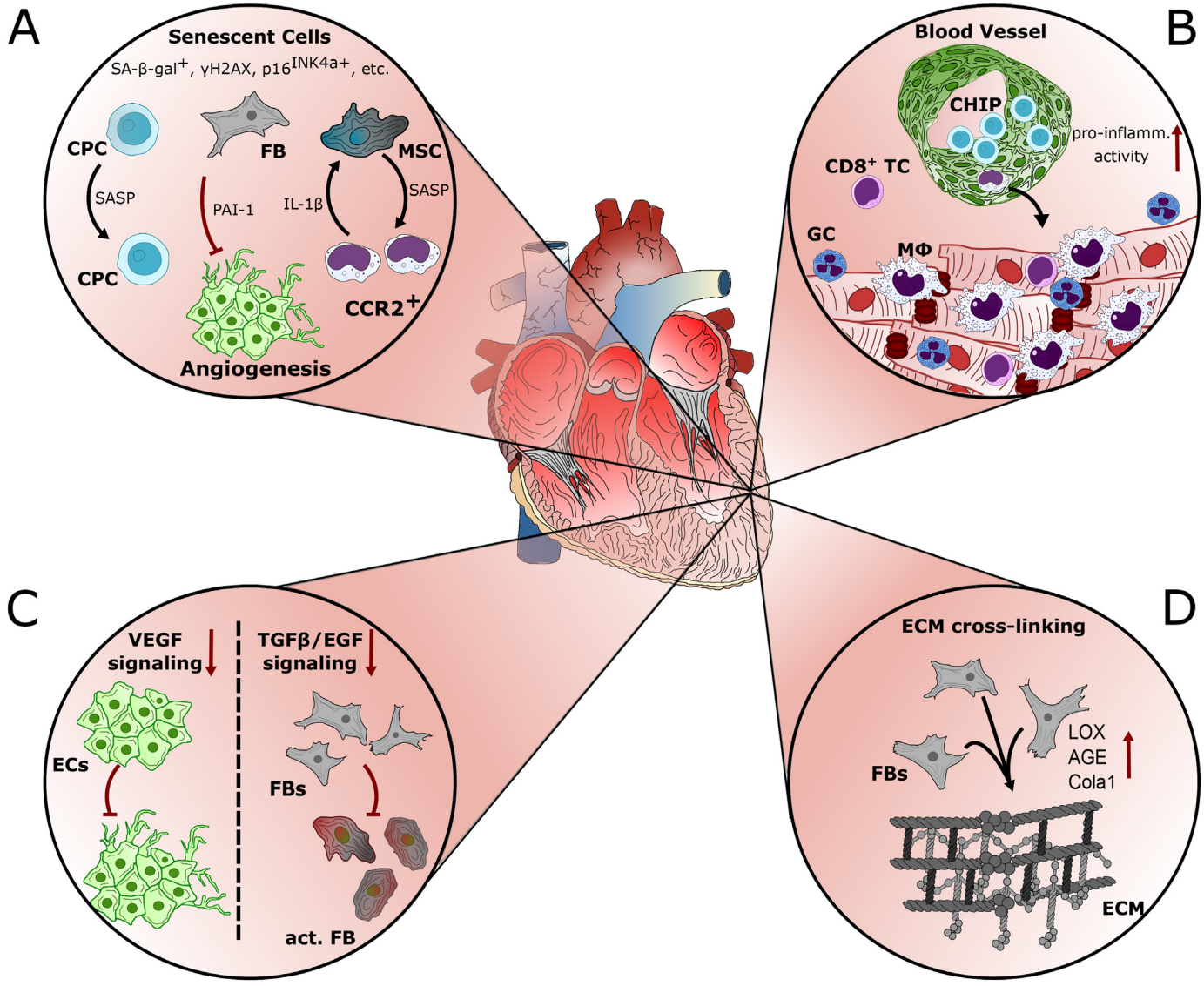

Fig. 3. Cellular cross-talk in the aging heart. (**A**) Cellular senescence is characterized by the presence of various biomarkers such as high senescence-associated β-galactosidase activity (SA- β-gal$^+$), γH2AX, p16^{INK4a} expression (p16^{INK4a+}), etc. Senescent cardiac progenitor cells (CPC) release SASP factors like PAI-1, IL-1, IL-6 and IL-8 and render senescence in otherwise healthy CPC. Aged cardiac fibroblasts (FB) secrete the SASP factor PAI-1 to inhibit angiogenesis and senescent mesenchymal stromal cells (MSC) release SASP factors that recruit CCR2$^+$ monocytes that release IL-1 β and further render senescence in MSC. (**B**) Ageing is associated with major alterations of the immune system, referred to as "inflamm-aging". This is reflected by alterations in the relative numbers of cardiac leukocytes: old hearts have proportionally more monocyte-derived cardiac macrophages (MΦ) and an increased population of granulocytes (GC). Moreover, aged mouse hearts have more CD8$^+$ T cells (TC) than other lymphocytes. (**C**) In addition, aging is generally associated with an impairment of receptor-mediated functions, because of the loss of receptors or their uncoupling from their specific signaling pathways. This is true for FBs, which show an impaired TGFβ and EGF response and endothelial cells, which are characterized by compromised VEGF signaling. (**D**) Aging FBs display increased extracellular matrix (ECM) production and an increased expression of lysyl oxidase (LOX) as well as advanced glycation end-products (AGE) that excessively cross-link collagens and thereby promote ventricular rigidity.

IL-1, IL-6, IL-8), ROS, matrix metalloproteases and urokinases, or FB-derived collagens (e.g. Collagen type 1a), which can all act in an auto- or paracrine fashion on neighboring cardiac cells (for review see [121,122]). The aged human heart was shown to contain an increased proportion of senescent cardiac progenitor cells that displayed high levels of the senescent biomarkers SA-β-gal, γH2AX and p16^{Ink4a}. As described for other senescent cells, these cells expressed also high amounts of SASP factors such as PAI-1, IL-8, IL-1β, MMP-3, GM-CSF and IL-6 that negatively impacted surrounding cells and promoted senescence of other cells to switch to a senescent phenotype, thereby reducing cardiac repair during aging [123]. Also FB of the aged heart secrete SASP associated factors. Single nucleus sequencing revealed that cardiac FBs of the aging heart highly express the SASP factor PAI-1. PAI-1 was further identified as one of the crucial factors in conditioned medium from age-heart derived FB, which interferes with endothelial cell angiogenesis [89]. In addition, mesenchymal stromal cells display senescence and SASP in the aging heart, which contributes to the recruitment of CCR-2-dependent monocytes. The recruited monocytes were associated with increased inflammatory macrophage-derived IL-1β, which in turn promoted further cardiac mesenchymal stromal cell senescence [124]. Interestingly, also systemic interactions control cardiac senescence. In parabiosis models it was shown that young mice can provide cardiac protection for aged mice [125]. While the origins of the cardioprotective effectors are not fully understood, a recent study suggests a contribution of adipose tissue [126]. Visceral adipose tissue was reported to increase osteopontin (OPN) expression during aging, which was associated with cardiac fibrosis and reduced cardiac function. Both the removal of visceral adipose tissue and OPN-deficiency in

aged mice restored cardiac function and reduced cardiac fibrosis. Interestingly, conditioned medium of OPN deficient adipocytes promoted FB senescence thereby limiting their expansion, indicating that FB senescence might also be a mechanism to protect from cardiac fibrosis [126]. But overall, targeting senescent cells in the aging heart might be a promising therapeutic strategy. In this respect, genetic removal of $p16^{Ink4a+}$ senescent cells by a *INK-ATTAC* transgenic mouse line extended lifespan and attenuated cardiac deterioration [127]. To be applied to patients, a new class of drugs was identified targeting specifically senescent cells, the so-called **senolytics**. This class of agents including dasatinb quercetin and the anti-cancer drug ABT263 have been used in various experimental models [128–130] and are being tested in proof-of-concept clinical trials to clear senescent cells from the aging tissue [131]. In a small Phase I study with patients with idiopathic pulmonary fibrosis, hints of improved physical functions were reported after treatment with dasatinb and quercetin, but the treatment was also associated with adverse events [131].

3.2. Immune cells in the aging heart

Ageing is additionally associated with major alterations of the immune system, referred to as **"inflamm-aging"** (Fig. 3B). This is reflected by alterations in the relative numbers and proportions of cardiac leukocytes: old hearts have proportionally more monocyte-derived cardiac macrophages and an increased population of granulocytes [21]. Moreover, aged mouse hearts have more $CD8^+$ T-cells than other lymphocytes, but the potential implications of these specific changes remain unclear (for review see [22]). In addition, hematopoietic stem cells can acquire mutations that lead to the expansion of mutated clones (so called clonal hematopoiesis of indeterminate potential, CHIP) leading to changes in the inflammatory properties of the mutated cell [132,133]. Such mutations predominantly occur in epigenetic regulators (such as *DNMT3a* or *TET2*) and are associated with detrimental effects of heart function in mice injury models [133,134] and prognosis of patients with aortic stenosis [135] or heart failure [136]. To what extend and how these mutations affect the local paracrine pattern and influence cardiac cells remains to be investigated.

3.3. Cardiomyocytes in the aging heart

With advancing age, the myocardium undergoes degenerative alterations [121]. This includes in particular a decrease in β-adrenergic receptor (β-AR) response that has been described by a process called "β-adrenergic receptor desensitization" by which phosphorylation of receptor structures results in a reduction in β-AR density and their internalization in the cell membrane [137,138]. Since β-AR stimulation triggers intercellular Ca^{2+} release from the sarcoplasmatic reticulum and thereby the activation of contractile proteins, the age-related decrease in β-AR density has a negative ionotropic effect that leads to a reduced ventricular function [137]. Cardiac aging is also associated with mitochondrial dysfunction with an increased ROS generation and release causing mitochondria DNA damage and hence further mitochondria dysfunction [139,140]. Furthermore, elevated levels of angiotensin II has been shown to induce CM hypertrophy, apoptosis as well as cardiac fibrosis directly [141]. The long-term inhibition of the angiotensin II converting enzyme and the angiotensin II receptor were both shown to prevent age-related cardiac pathologies and prolonged rodent survival [142].

Further factors, which are involved in cardiac aging include (but are not limited to) IGF-1 and GDF-11 which are both decreased on circulating level. GDF-11 (= growth differentiation factor-11, a member of the TGFβ family) shows a decreased blood level in cardiac aging. By combining young and aged mice in a parabiosis study, Loffredo et al. restored circulating GDF-11 blood levels that prevented age-related cardiac hypertrophy and a reduced expression of the hypertrophy-related marker genes ANP and BNP in CMs [125]. In humans, low circulating levels of GDF-11 were associated with increased ventricular hypertrophy [143]. However, the findings have been controversial. Smith et al. injected recombinant GDF-11 in 24-months-old mice to restore circulating GDF-11 levels and were unable to reverse cardiac hypertrophy. Cardiac structure and function were also not restored. By using phenylephrine-treated neonatal rat CM, GDF-11 did not prevent but rather promoted hypertrophy *in vitro* [144]. Likewise, IGF-1 appears to have also a controversial role in cardiac aging: both protective and detrimentally effects have been described in the aging heart. Several studies demonstrated that IGF-1 acts anti-inflammatory and has a cardio-protective functions [145]. In addition, IGF-1 deficient mice showed an increased life expectancy and the same association was seen in human [146,147]. However, some studies show contradicting results. Whereas IGF-1 overexpression in mouse hearts showed reduced CM death after MI and reduced ventricular dilation, hypertrophy, as well as diabetic cardiomyopathy [148–150], other studies demonstrated that IGF-1 overexpression causes cardiac hypertrophy and heart failure and worsen recovery upon MI [151,152]. Additionally, age-dependent increased expression of the inflammatory mediator ANGPTL2 (angiopoietin-like protein 2) is associated with cellular senescence (so far only proven in the skeletal muscle [153]) and its overexpression in the heart impairs cardiac function by decreasing myocardial energy metabolism and inactivation of Akt as well as sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)2a signaling [154] suggesting that it might also contribute to cardiac dysfunction in the aging heart.

3.4. Fibroblasts in the aging heart

Cardiac aging is associated with an increase in fibroblasts relative to other cell types of the heart [155]. Cardiac fibrosis is a reactive phenomenon that is associated with left ventricular hypertrophy [156]. However, the underlying mechanisms mediating fibrosis during aging are still not fully understood. It is known that elevated angiotensin II levels and chronic inflammation can increase FB activation, proliferation, matrix protein production and hypertrophy [156]. Furthermore, aged cardiac FB were demonstrated to increase the expression of prolyl hydroxylases and lysyl oxidase that promote ventricular stiffness by cross-linking matrix protein chains [157,158].

Additionally, aging is generally associated with an impairment of receptor-mediated functions, because of the loss of receptors or their uncoupling from their specific signaling pathways [159]. This is true for FBs, which show an impaired TGFβ and EGF response [160,161] and ECs, which are characterized by compromised VEGF signaling [162] in aging (Fig. 3C-D). One may speculate that a more general decline in receptor-mediated signaling may compromise the cellular interactions and fine tuned communication in the heart.

3.5. Endothelial cells in the aging heart

Aging plays a major role in vascular and cardiac remodeling. With advancing age, endothelial senescence and inflammation is mediated via the above mentioned pathways including oxidative stress, telomere erosion and mitochondrial dysfunction [163]. FOXO and SIRT are two major regulators that control endothelial function [164]. Whereas overexpression of FOXO1 in mice shows reduced angiogenesis [165], FOXO3 and SIRT1 were described to regulate the expression of genes involved in mitochondrial antioxidant defense (MnSOD, catalase, sestrins, and selenoprotein P) and DNA repair (GADD45a), thus, opposing the age-related effects of ROS in ECs [166–168]. Since both FOXO and SIRT are downstream targets of IGF-1 and given that FOXO and SIRT play a protective role in maintaining health and function of ECs, they could be potential players in the age-related decline of endothelial function.

Similar to FBs, aging may also control extracellular matrix production in ECs. A dysregulation of the laminin β1 and β2 chain expression in aged cardiac mouse ECs was previously described. In the

aging heart, ECs shift the expression from laminin β2 to β1 and thereby modulate cell-matrix adhesion, cell migration and EndoMT in an autocrine manner that may impair vessel integrity [169]. Endothelial integrity is further disturbed by the age-related increase in ROS in the vasculature that leads to a Src-dependent degradation and loss of vascular-endothelial (VE)-cadherin from adherens junctions which impairs the ability to amplify endothelial dilation [170].

Advanced age is also associated with deteriorations of the arterial system that contribute to organ and tissue dysfunction [171]. Aging central arterial ECs display a decreased NO production [172,173], impaired barrier function [174] and are more prone to apoptosis [175]. They additionally have a pro-inflammatory phenotype which includes the expression of various pro-inflammatory mediates such as ET-1 [176], angiotensin II [177], TGFβ, TNFα and IL-6 [178]. This pro-fibrotic and pro-inflammatory environment can be permissive for EndoMT and hence contribute to pathophysiologic alterations of the arterial wall like neointima formation and atherosclerosis. Evidences are given by studies showing that aged arterial ECs reduce internalization but increase degradation of VE-cadherin [170,179]. Furthermore, aging arterial ECs reveal a prominent intimal fibrous lesion with expression of rigid proteins such as collagen and fibronectin. Vascular stiffness is further promoted by reduced compliance of elastic fibers and extensive cross-linking of collagen and fibronectin chains [180–182]. The expression of the pro-fibrotic and pro-inflammatory factors mediating these processes can be localized to ECs and smooth muscle cells (SMC). In vitro assays showed that SMCs from aged arteries have increased proliferative and migratory capacities as well as higher NFkB activity and expression of pro-fibrotic and pro-inflammatory mediators compared to young arterial SMCs. In contrast, mature contractile SMCs might have a reduced viability in aging arteries [180–183]. As the aged arterial wall provides a pro-inflammatory environment that might support EndoMT, ECs undergoing EndoMT and not solely SMCs might contribute to arterial stiffness and fibrosis and hence promote arterial dysfunction [171].

4. Perspectives

The presented examples illustrate many facets of intracellular communication, which can be essential for maintenance of tissue homeostasis, repair and regeneration but may also contribute to pathological processes in the heart. Most studies so far focused on the elucidation of well-known mediators of cellular communication, mainly connexins, soluble cytokines or growth factors which act on receptor expression in other cell types or other ligand-receptor interaction. Other ways of communication, e.g. microRNAs or other RNAs by exosomes, have also been studied, but mainly as therapeutic vehicles; their role as natural communication tools in physiological settings in tissues is less clear. Advanced technologies are likely to provide novel insights into small metabolites or lipids which can control cell-cell communication and influence cardiac physiology and disease. Finally, we are just at the beginning to understand the cellular heterogeneity within given cell types. Here single cell technologies likely will provide insights into the changes of cellular phenotypes under disease setting and may help to gain a deeper understanding of the impact of disease and aging on cellular phenotypes. All of these new insights may provide targets for controlling pathologically relevant alterations of cellular communication pathways in the future.

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