REVIEW

Endothelial-Mesenchymal Transition or Functional Tissue Regeneration – Two Outcomes of Heart Remodeling

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Summary

Heart remodeling occurs as a compensation mechanism for the massive loss of tissue during initial heart failure and the consequent inflammation process. During heart remodeling fibroblasts differentiate to myofibroblasts activate their secretion functions and produce elevated amounts, of extracellular matrix (ECM) proteins, mostly collagen, that form scar tissue and alter the normal degradation of ECM. Scar formation does replace the damaged tissue structurally; however, it impedes the normal contractive function of cardiomyocytes (CMs) and results in long-lasting effects after heart failure. Besides CMs and cardiac fibroblasts, endothelial cells (ECs) and circulating endothelial progenitor cells (cEPCs) contribute to heart repair. This review summarizes the current knowledge of EC-CM crosstalk in cardiac fibrosis (CF), the role of cEPCs in heart regeneration and the contribution of Endothelial-mesenchymal transition (EndoMT).

Key words

Cardiac fibrosis • Endothelial-mesenchymal transition • Endothelial progenitor cells • Cardiac fibroblasts • Cardiomyocytes • Endothelial cells • Agiotensin II • Tumor Growth Factor-β

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Introduction

Cardiovascular disease is the main cause of death in developed countries worldwide. Cardiac dysfunction is the primary consequence of cardiac remodeling, caused by the hypoxic and inflammation response during heart ischemia. Approximately 50 % of patients diagnosed with cardiac dysfunction will die within five years. In addition, 40 % of patients die within one year after hospitalization for cardiac failure (Liu and Eisen 2014). A significant part of deaths associated with cardiac remodeling and dysfunction is caused by sudden death, which suggests that even though a patient is asymptomatic it does not guarantee of good prognosis (Pimentel *et al.* 2014). Despite the increased survival with modern therapies, mortality rates are still at extremely high levels (Braunwald 2013).

The tissue injured by heart ischemia and massive cell death requires a relatively rapid process to compensate for cellular loss and activates regeneration and remodeling processes. Even though these reparation processes quickly compensate for the structural damage, they are not able to replace the functionality of the tissue, which has long-term effects. The regulation of the tissue regeneration and remodeling could be beneficial for partial recovery of the function; however, any treatment intervention must be preceded by detailed knowledge of the molecular processes in place.

Most studies focus on the CMs' response to heart ischemia as a cause of CF. The role of ECs in the process has been neglected, even though they are a major contributor to the myofibroblast pool (Pardali *et al.*

2017). In heart failure patients without coronary artery disease, coronary endothelial dysfunction correlates with adverse cardiac remodeling and contractile abnormalities; however, the endothelial function of peripheral arteries is preserved. This suggests that localized endothelial dysfunction and crosstalk between CMs and ECs are responsible for cardiac remodeling and hypertrophy.

EC-CM crosstalk guides CF

In the adult heart, capillaries are densely distributed throughout the myocardium, and the number of ECs is approximately 3 times higher than CMs. Together, CMs and ECs form their physiological niche by secreting of a large number of signaling molecules that affect the surrounding cells and facilitate the homeostasis of the tissue.

CMs have a significant effect on ECs' function during cardiac remodeling. They are a major player in angiogenesis and neovascularization during heart repair. CMs are the main contributor to the vascular endothelial growth factor (VEGF) pool which is the key controller of angiogenesis (Colliva *et al.* 2020, Giordano *et al.* 2001). Despite the fact that CMs constitute less than one-third of the total number of cells in the heart, the CM-specific deletion of VEGF gene decreases the total mRNA levels by more than 85 %.

Recent data also show that CMs directly stimulate the angiogenesis of resident ECs by paracrine stimulation in response to cardiac remodeling signaling (Zhang *et al.* 2021b). In a report by Gladka and colleagues CMs activated the Zinc finger E-box-binding homeobox 2 (ZEB2) expression in response to remodeling stimulation, which resulted in an increase of circulating levels of Tymosin β4 (TMSB4) and Prothymosin α (PTMA). Both TMS4 and PTMA have been linked to cardioprotection by regulation of neovascularisation, angiogenesis, and apoptosis (Gladka *et al.* 2021, Smart *et al.* 2010). It was shown that TMSB4 stimulates the formation of new CMs, originating from the epicardium, and promotes neovascularization (Malinda *et al.* 1998, Tang *et al.* 2021). PTMA has also been shown to stimulate cardiac EC migration, angiogenesis, and wound healing (Halder *et al.* 2020). Interestingly, ZEB2 was also shown to initiate EndoMT in carcinogenesis, which suggest that regulation of ZEB2 signaling is crucial for the balance between heart regeneration and fibrosis (Comijn *et al.* 2001).

ECs, on the other hand, control CM contractility by a number of growth factors. During heart inflammation and heart remodeling, EC-CM communication guides tissue fibrosis by regulation of nitric oxide (NO) bioavailability and Angiotensin converting enzyme 2 (Ace2) production (Han *et al.* 2018, Heiss *et al.* 2015). NO is one of the most significant signaling molecules regulating vascular function and cell recruitment during injury. It is synthetized by the NO synthase class of enzymes (eNOS). Different eNOS types are either constitutively active and produce NO in response to calcium, or inducible by cytokine stimulation in response to tissue injury. In the adult heart, NO is mainly produced by ECs and controls vascular smooth muscle cells, and the onset of ventricular relaxation, thus optimizing pump function at every cardiac contraction (Heiss *et al.* 2015). eNOS catalytic activity requires the cofactor tetrahydrobiopterin (BH4). Reactive oxygen spieces (ROS) are one of the major regulators of eNOS activity through oxidation of BH4 to 7,8-dihydrobiopterin (BH2) (McNeill and Channon 2012). BH2 changes eNOS enzymatic activity, which produces superoxide in place of NO. This mechanism decreases NO bioavailability and significantly contributes to fibrosis by the dysregulation of vasoconstriction and NO signaling for stem cell recruitment discussed later.

Angiotensin II (AngII) is a major inducer of CF *in vitro* and only recently AngII inhibition was shown to have a cardioprotective effect in patients with hypertension-induced heart failure (Huang *et al.* 2020). The level of AngII in heart is strictly controlled by hydrolysis by Ace2 (De Mello and Danser 2000). Ace2's analog Ace, however, does not share this functionality and Ace2-to-Ace ratio in circulation is responsible for the control of AngII levels (Fig. 1). This delicate physiological balance is disrupted during cardiac stress, when EC expression of Brahma-related gene-1 chromatin remodeler (Brg1) and forkhead box M1 (FoxM1) transcription factors cause the formation of a protein complex at the Ace/Ace2 promoter site, followed by concurrent activation of Ace and repression of Ace2. This shifts the Ace-to-Ace2 ratio balance in favor of Ace, resulting in AngII production. Subsequently, AngII stimulates fibrosis in two phases (Yang *et al.* 2016). In the early phase of CF, AngII stimulates direct association between the Toll-like receptor 4 (Tlr4) and Signal transducer and activator of transcription 3 (STAT3) (Han *et al.* 2018). Phosphorylated STAT3 activates its transcription targets including interleukin-6 (IL-6), resulting in increased IL-6 production, which induces late phase of STAT3 transcription and its targets including the transforming growth factor β (TGF-β). IL-6 and TGF-β are notorious inducers of cardiac remodeling.

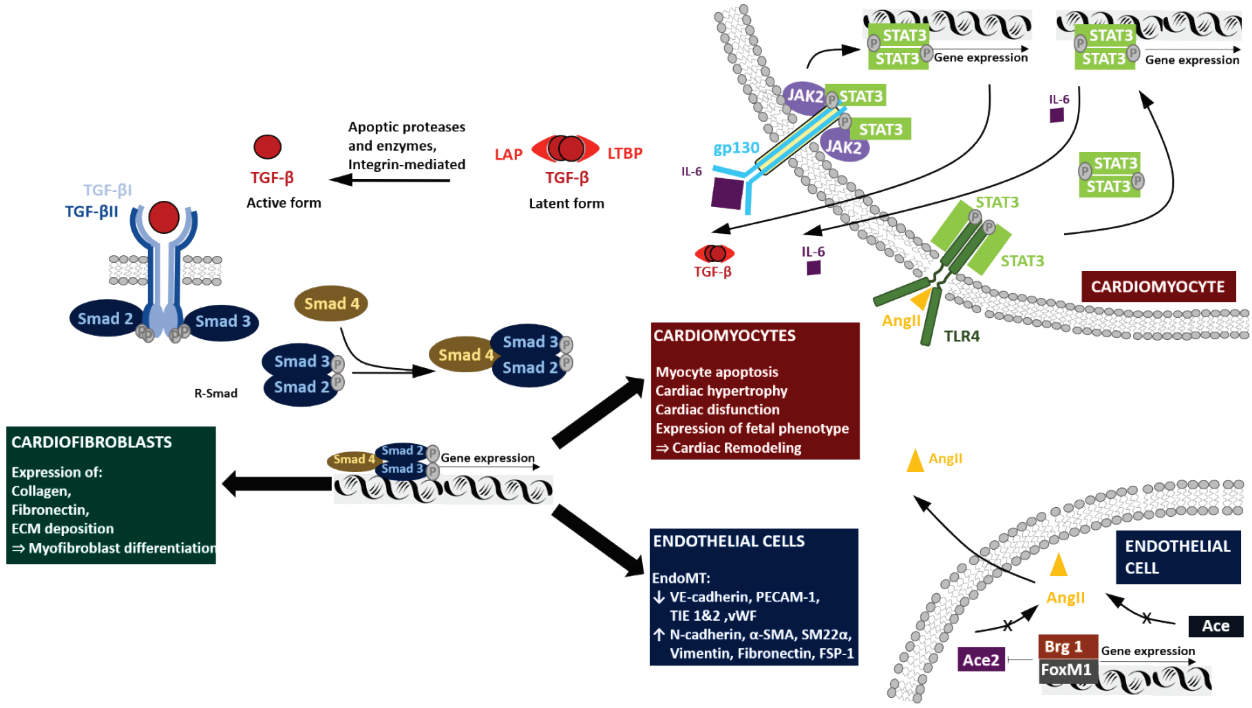

Fig. 1. CM-EC crosstalk during heart remodeling. During heart remodeling complex Brb1/FoxM1 modifies the transcription of Ace and Ace2 in ECs, which prevents degradation of AngII. In result, AngII activates cardiomyocyte TLF4 receptor, which phosphorylates STAT3, which initiates transcription of multiple targets including IL-6. IL-6 is further secreted into extracellular space, binds to gp130 receptor and activates secondary STAT3 targets including TGF-β. TGF-β is again secreted to extracellular space and activates transcription of TGF-β targets trough Smad signaling pathway in cardiofibroblasts, CMs and ECs.

The production of TGF-β alone is however not solely responsible for the activation of the intracellular signaling cascade (Gentry and Nash 1990, Robertson and Rifkin 2016). TGF-β is secreted in a latent form that contains the TGF-β dimer, the latency-associated peptide (LAP), and a latent TGF-β-binding protein (LTBP), which sequesters the complex into the ECM (Fig. 1). The activation mechanisms of TGF-β are currently unknown. A list of possible suspects includes ROS, ECM protein interactions, and, most recently, tissue nonspecific alkaline phosphatase (Cheng *et al.* 2021, Hanna and Frangogiannis, 2019). TGF-β activation initiates the Smad2/3 pathway, promotes the process of CF and induces the transformation from fibroblasts into myofibroblasts (Desmoulière *et al.* 1993, Heldin *et al.* 1997, Shi and Massagué 2003). Furthermore, TGF-β increases ECM gene expression, promotes ECM deposition and inhibits the degradation of ECM by regulating the level of plasminogen activator inhibitor and tissue inhibitors of metalloproteinases (Leask 2010, Schiller *et al.* 2004). TGF-β also affects the CM phenotype, where TGF-β stimulation results in the hypertrophy of CMs (Lim *et al.* 2005). This finalizes the process of scar tissue formation.

The role of EndoMT in cardiac regeneration and CF

EndoMT is a complex biological process where ECs lose their specific cell profile, decrease vascular endothelial cadherin expression, and acquire a myofibroblast phenotype characterized by smooth muscle actin, vimentin, and type I collagen expression. Besides the acquisition of an activated pro-fibrogenic phenotype, these cells also increase their mobility and migrate to surrounding tissues.

TGF-β was shown to have more diverse effects than fibroblast transformation. Epithelial, endothelial and immune cells were shown to contribute to the total myofibroblast pool in a fibrotic heart by epithelial/endothelial-to-mesenchymal transition in response to TGF-β (Pardali *et al.* 2017, Xu *et al.* 2009, Zeisberg *et al.* 2007). In CF, EndoMT represents the most important contributor to the generation of fibrotic tissue (Piera-Velazquez *et al.* 2011). Moreover, the inhibition of TGF-β signaling prevented epithelial-to-mesenchymal transition (Meng *et al.* 2012). ECs themselves were also shown to contribute to TGF-β production and secretion under hypoxic conditions, which, in turn, promotes EndoMT in an autocrine loop and induces apoptosis in

CMs (Sniegon *et al.* 2017). It appears that EndoMT and functional tissue regeneration are the opposite outcomes of a common process. Close optimization of the growth factor composition of the specific niche where regeneration and fibrosis take place may represent a novel target for the prevention of tissue fibrosis.

The role of cEPCs in heart remodeling

The contribution of bone-marrow derived stem cells to heart regeneration has been debated since the discovery of cEPC's and mesenchymal stem cell's differentiation abilities. EPCs are a bone marrow stem cell population, considered a remnant of developmental hemangioblast characterized by CD34, CD31, VEGF receptor 2 and CD133 expression. These progenitor cells show limited stem cell features, such as clonal expansion and angiogenic capability. A low number of EPCs is constitutively present in human blood circulation. They account for only 0.001 %-0.0001 % of peripheral blood cells in a physiological state (Asahara *et al.* 2011). Cardiac injury and inflammation signaling mobilizes EPC's from blood marrow into circulating blood and homing to the injury site. The question of cEPC's contribution to tissue repair and regeneration is controversial. It was shown that cEPCs contribute to tissue repair by paracrine signaling, and their differentiation into mature ECs and integration into the vessel wall was shown repeatedly as well. However, the extent of their contribution was repeatedly questioned.

The correlation between the number of cEPC and the state of the cardiovascular system is an appealing argument in favor of cEPCs' role in cardiac regeneration. The enumeration of cEPCs is a diagnostic parameter that reflects the condition of the vascular network, and their number decreases in multiple diseases that affect the cardiovascular network like diabetes and artherosclerosis (Chironi *et al.* 2007, Fadini 2014). Hill's assay evaluates patients with different cardiovascular risk factors dependent on the number of cEPCs. A recent study showed a correlation between the cEPC count, severity of coronary artery disease and cardiovascular risk (Hill *et al.* 2003, Zhang *et al.* 2021a). It was shown that cEPCs contribute to endothelial repair at the moment of endothelial injury, and their depletion or decrease has a detrimental effect on injury response and tissue regeneration (Kocher *et al.* 2001, Zhang *et al.* 2021a). It was demonstrated that neoangiogenesis by cEPCs inhibits the apoptosis of hypertrophied myocardium, minimizes abnormal collagen deposition and scar formation, and optimizes ventricular function (Kocher *et al.* 2001, Wang *et al.* 2021). A recent study found that cardiac resynchronization therapy increases cEPC count and improves cardiovascular outcome (Cristóvão *et al.* 2020, Yang *et al.* 2020). As a previous study showed IL-6, as a major biomarker of inflammation response, is reduced after 3 months of biventricular pacing (Theodorakis *et al.* 2006). Therefore, increased cEPC mobilization can be directly linked to a decrease in inflation signaling.

EPC mobilization during heart remodeling is, however, obstructed. Endothelium-derived NO is a major regulator of both EPC mobilization from bone marrow and its function. (Aicher *et al.* 2003, Sandri *et al.* 2016). Due to the low bioavailability of NO during heart remodeling EPC mobilization is limited (Chironi *et al.* 2007, Samman Tahhan Ayman *et al.* 2017). A recent study showed the recovery of EPC mobilization through the activation of eNOS with fenofibrate (Huang *et al.* 2021). Furthermore, the half-life of EPCs is also reduced in heart failure, which further drives heart remodeling and adverse outcomes (Samman Tahhan Ayman *et al.* 2017).

Although the differentiation of cEPCs into CMs was repeatedly demonstrated *in vitro*, it still remains controversial (Bachelier *et al.* 2020, Badorff *et al.* 2003, Koyanagi *et al.* 2005, Murasawa *et al.* 2005). The reason is that this process was never proven to contribute to heart regeneration *in vivo*. Resident cardiac ECs were recently shown to express CM myofibril (CMF) genes and have open chromatin at CMF gene promoters. However, these open chromatin signitures disappeared at EC cultivation *in vitro* (Yucel *et al.* 2020). These data demonstrate that ECs maintain fully open chromatin at CM-specific genes, which suggests certain phenotype plasticity. The fact that cEPCs and resident cardiac ECs contribute to fibrosis by EMT and were also shown to transdifferentiate into functional CMs raises the possibility that the specific CM-EC niche can be modulated to favor of differentiation into partially functional heart tissue.

Conclusion

ECs significantly contribute to cardiac remodeling and fibrosis. It appears that the remodeling program that takes place in the environment of inflammation signaling favors the highly proliferating fibroblast population that creates scar tissue and reduces the functionality of the cardiac tissue. Moreover, cells

with regenerative capabilities and cardiac trans-differentiation potential are affected by the paracrine signaling and go through EndoMT transition. Shifting the delicate balance of CM-EC crosstalk to favor angiogenesis and trans-differentiation of ECs and EPCs into CMs instead of EndoMT could significantly improve the tissue function. The modulation of the CM-EC specific cardiac niche is an interesting new target for CF therapy.

Conflict of Interest

There is no conflict of interest.

Abbreviations

Brg1, Brahma-related gene-1 chromatin remodeler; FoxM1, forkhead box M1; TIMPS, tissue inhibitors of metalloproteinases; Ace, Angiotensin converting enzyme Ace2, Angiotensin converting enzyme 2; AngII, angiotensin II; BH2, 7,8-dihydrobiopterin; BH4, tetrahydrobiopterin; cEPC, circulating endothelial progenitor cells; CF, cardiac fibrosis; CMF, cardiomyocyte myofibril; CMs, cardiomyocytes; ECM, extracellular matrix; ECs, endothelial cells; EndoMT, endothelial- mesenchymal transition; eNOS, NO synthase class of enzymes; IL-6, interleukin 6; NO, nitric oxide; PTMA, Prothymosin α; ROS, reactive oxidative species; STAT 3, Signal transducer and activator of transcription 3; TGF-β, transforming growth factor β; Tlr4, Toll-like receptor 4; TMSB4, Tymosin β4; TNF-α, tumor necrosis factor α; ZEB2, Zinc finger E-box-binding homeobox 2

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