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Cardiac fibrosis – A short review of causes and therapeutic strategies

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ABSTRACT

Fibrotic diseases cause annually more than 800,000 deaths worldwide, whereof the majority accounts for lung and cardiac fibrosis. A pathological remodeling of the extracellular matrix either due to ageing or as a result of an injury or disease leads to fibrotic scars. In the heart, these scars cause several cardiac dysfunctions either by reducing the ejection fraction due to a stiffened myocardial matrix, or by impairing electric conductance, or they can even lead to death. Today it is known that there are several different types of cardiac scars depending on the underlying cause of fibrosis. In this review, we present an overview of what is known about cardiac fibrosis including the role of cardiac cells and extracellular matrix in this disease. We will further summarize current diagnostic tools and highlight pre-clinical or clinical therapeutic strategies to address cardiac fibrosis.

Contents

1. Introduction

Cardiovascular disease (CVD), a class of diseases that impact the heart or cardiovascular system, is responsible for 31% of all deaths and remains the leading cause of mortality worldwide [1]. Ischemic heart disease and endomyocardial fibrosis are the primary causes of end-stage heart failure [2]. In 2012, 1 of 9 deaths in the US was due to heart failure. According to the American Heart Association, the estimated annual costs for CVD and strokes are $316.6 billion per year, more than any other diagnostic group [2]. The estimated costs for CVD in Europe are 210 billion € per year [3]. There is an urgent need to develop advanced diagnostic tools and improved therapies in the area of cardiac disease and especially cardiac fibrosis.

Fibrosis is a well-recognized cause of morbidity and mortality [1]. Fibrotic scars of the cardiac muscle most commonly occur after myocardial infarction; however, there are various other conditions promoting cardiac fibrosis such as hypertensive heart disease, diabetic hypertrophic cardiomyopathy and idiopathic dilated cardiomyopathy [4,5]. Despite the impressive self-healing capacity of the human body, where small defects as a result of injury or disease can be remodeled or regenerated by the residing cells, not all defects can properly regenerate, which is especially true for the human heart. Cardiac fibrosis is a process of pathological extracellular matrix (ECM) remodeling, leading to abnormalities in

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matrix composition and quality, as well as an impaired heart muscle function [4]. Initially, ECM deposition is a protective mechanism and can be beneficial for wound healing and tissue regeneration. However, excessive and continuous ECM deposition, particularly collagen type I secretion, leads to impaired tissue function [1].

2. ECM and ECM of the cardiac muscle

Tissues and organs consist of two main elements: A non-cellular ECM component, and residing cells, which constantly synthesize or degrade the ECM [6]. The ECM is a highly durable, mechanically stable fiber-containing structure that serves as a scaffold for cells. Cell-ECM interactions influence cellular behaviors such as migration, proliferation, survival and differentiation, for example via cell-surface receptors [6–9]. Each tissue has unique mechanical and biological properties that are defined by a complex network of collagens, elastic fibers, fibronectin, laminins, various proteoglycans, glycosaminoglycans, glycoproteins and soluble factors [6,10]. The exact composition of the ECM varies from tissue to tissue. The major component of the adult human cardiac muscle is fibrilliar collagen type I (approximately 85%) and collagen type III (approximately 11%) [11]. Collagen synthesis and turnover is mainly regulated by cardiac fibroblasts [12]. In the heart muscle, collagen fibers are highly important for the transmission of contractile forces [12]. Collagen fiber diameters vary depending on their location. A fiber diameter between 120 nm and 150 nm has been reported for collagen fibers in the endomysium [13], the region surrounding individual cardiomyocytes [14]. Two diameter ranges were described for the perimysium, the region defining muscle bundles, which contains collagen fibers between 1 and 3 μm and 40–50 nm in diameter [14,15]; however, these parameters significantly change based on age or physiological condition [16]. In addition to collagens, the cardiac muscle also contains elastic fibers, fibronectin, proteoglycans and glycosaminoglycans [11,14]; although the ECM composition and quality changes dramatically in aged [6] or diseased tissues [17] (Fig. 1). Based on many studies much is known about the composition of the cardiac muscle in health and disease; however, there is still a lack of understanding of the molecular mechanisms that underlay the interaction of cellular and extracellular components of the cardiac muscle [14].

3. Cells in the cardiac muscle

The cardiac muscle is composed of various cell types. Cardiomyocytes are commonly known as the cells of the heart; although only 25–35% of all cells in the adult cardiac muscle are cardiomyocytes [18]. During early heart development, cardiac progenitor cells differentiate into cardiomyocytes, which then start to proliferate [19]. Fetal heart growth is a result of cardiomyocyte proliferation; however, in an adult heart, cardiomyocyte turnover is highly limited if even existing. Shortly after birth the cells exit the cell cycle, and it has been reported that the turnover rate in the adult heart is less than 1% a year [19].

Specific markers to identify cardiomyocytes are sarcomeric myosin (MF20), cardiac troponin (cTNT) and connexin 43 (Cx43) [20]. Ventricular and atrial cardiomyocytes express the contractile proteins MF20 and cTNT; however, they differ in their contractile and electrophysiological properties [21,22]. A few genes have been identified to separate atrial and ventricular cardiomyocytes: HRT1, MLC-2a, CX40, sarcolipin and ANF were described to be expressed in the atrium, whereas HRT2, IRX4, MLC-2v, KCNE1 are expressed in the ventricle [22]. Brauchle et al. used Raman microspectroscopy as a marker-independent method to distinguish between atrial and ventricular cardiomyocytes [21]. Compared to ventricular cadiomyocytes, atrial cardiomyocytes show an altered calcium signaling due to the lack of transverse tubules [23]. Furthermore, it has been described that sino-atrial node cells exhibit a different morphology than atrial or ventricular cardiomyocytes [23]. These reports show that cardiomyocytes are not homogeneous cell populations. Although it has been reported that the adult myocardium additionally contains cardiac stem cells, a multipotent cell population, which gives rise to cardiomyocytes and vascular cells when stimulated under the right conditions [24],the presence of a true multipotent cardiac stem cell in the adult human heart is still under heavy scientific debate.

There is a significant number of non-myocytes present in the heart. The non-myocyte cell population includes vascular and lymphatic endothelial cells, fibroblasts, vascular smooth muscle cells, pericytes, tissue macrophages and leucocytes (including myeloid cells, T cells and B cells) [25]. It has been reported that about 64% of the non-myocyte cell population in the mouse and 54% in the human heart are endothelial cells [25]. Furthermore, about 9% and 3% leucocytes have been identified in the mouse and human heart, respectively [25]. Until today, the exact quantity of cardiac fibroblasts in the human heart has not been examined; however, a study on adult mouse hearts confirmed that about 15% are cardiac fibroblasts. In the majority of the studies, cardiac fibroblasts were defined by their morphology, but there is a combination of cellular markers such as Ddr2, CD90, PDGFRα, vimentin, Fsp1, Tcf21, Col1a1, MEFSK4 and Postn, which have been used to identify these cells [25,26]. Cardiac fibroblasts are responsible for (physiological and pathological) ECM synthesis in the heart muscle and play an important role for the structural, mechanical and electrical cardiac function [26–28].

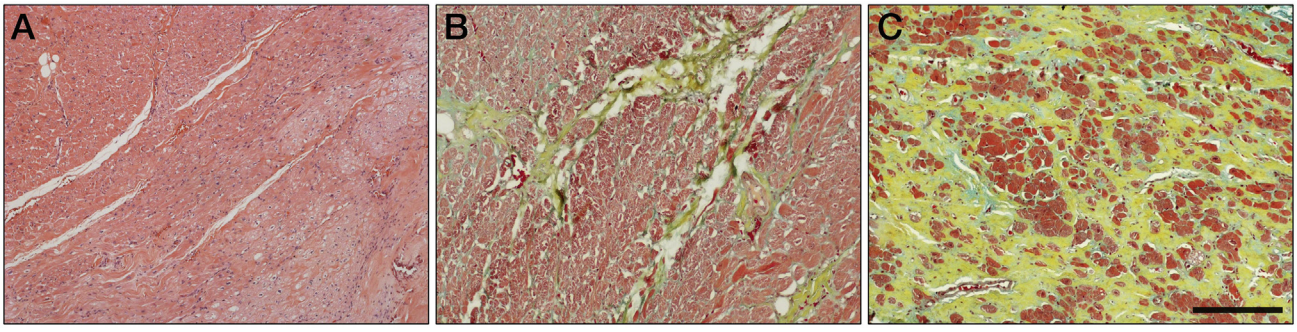

Fig. 1. Movat-Pentachrome staining of the adult human ventricle. (A) Healthy adult, (B) ischemic cardiomyopathic (interstitial fibrosis) and (C) dilated cardiomyopathic (diffuse fibrosis) muscle tissue. Muscle tissue is red, proteoglycans and glycoproteins are shown in blue-green, and collagens are depicted in yellow. Scale bar equals 200 μm.

4. Cardiac fibrosis

Cardiac fibrosis is a scarring event in the cardiac muscle that is characterized by an increased collagen type I deposition as well as cardiac fibroblast activation and differentiation into myofibroblasts [5]. These pathological changes lead to an increased matrix stiffness and lead to abnormalities in cardiac function. Three types of myocardial fibrosis have been identified: I) reactive interstitial fibrosis, II) infiltrative interstitial fibrosis and III) replacement fibrosis. Reactive interstitial fibrosis leads to a pressure overload and cardiomyopathies due to an increased ECM deposition without a significant loss of cardiomyocytes [5,29]. Infiltrative interstitial fibrosis is characterized by the glycolipid build up in different cells of the heart, which can be observed in patients with Fabry disease, a rare genetic disease that involves dysfunctional metabolism of sphingolipids [5,29]. Replacement fibrosis occurs after cardiac injury like myocardial infarction, where cardiac cells are damaged. Here, dead cells are replaced and a predominantly collagen type I-containing scar is formed [5,29]. The texture of fibrosis in the diseased heart varies strongly. Histological assessment utilizing collagen type I staining of human fibrotic hearts revealed four different types of fibrotic structures: interstitial, compact, diffuse and patchy [30]. Interstitial fibrosis is mainly an accumulation of collagens between groups of cells. Compact fibrosis is characterized by the deposition of large and dense collagenous structures, which are completely devoid of cardiomyocytes. Short stretches of fibrosis are typical for diffuse fibrosis, whereas patchy fibrosis shows long collagen fiber strands in between myocardial bundles [30].

4.1. Collagens in fibrosis

ECM remodeling and crosslinking occurs after injury as a result of wound healing or in the aged heart. Both events play a crucial role in cardiac fibrosis, which is pathologically characterized by an increased deposition of collagens that is induced by activated cardiac fibroblasts [30]. Additionally, an altered collagen type I to collagen type III ratio is attributed to cardiac fibrosis. The ratio of collagen type I to collagen type III varies depending on the underlying cause of fibrosis. In myocardial infarction (MI) models, an upregulation of collagen type I was observed [12,31], whereas the expression of type III collagen was significantly increased in patients suffering from ischemic cardiomyopathy [12,32]. The role of collagen type VI in cardiac fibrosis is not entirely understood; however, it has been shown that it can induce myofibroblast transdifferentiation [12]. Interestingly, the same study reported that the disruption of collagen type VI resulted in a reduction of fibrosis [12].

It has been reported that the accumulation of collagens followed by a maturation process leads to a fibrotic scar [1]. The tensile strength of such scars increases with crosslinking density [33]. As a result, cardiac contractility and relaxation are impacted and the cardiac function is limited [1]. Excessive collagen deposition in the heart muscle also effects electric coupling. Scars can create conduction blocks and have therefore been described as electrical insulators [34–36]. But is a fibrotic scar really non-conductive? Catheter ablation is a clinical intervention by which a tightly focused lesion is induced by a local energy delivery in order to correct atrial fibrillation by forming a scar to prevent undesired currents to pass through. Interestingly, in many patients these scars become transparent after a while, and it has been reported that there is a possibility of trans-scar conduction [36,37]. Future studies are needed to further the understanding of the biology and underlying molecular mechanisms that lead to functional scars in the heart, and how the quality of cardiac ECM components and their composition impact normal cardiac function. This knowledge will allow the development of novel cell-free treatment strategies for the fibrotic heart such as utilizing ECM proteins as a therapeutic agents.

4.2. The role of fibroblasts

Cardiomyocyte death after injury or due to diseases leads to an inflammatory response and activation of cardiac fibroblasts [12]. Under physiological conditions, cardiac fibroblasts express no stress fibers. After injury however, fibroblasts are activated and transdifferentiate into stress-fiber expressing myofibroblasts [12,36]. This phenotype expresses α smooth muscle actin and develops contractile bundles [38,39]. Changes in the mechanical and structural microenvironment but also the increase of certain factors such as transforming growth factor β (TGFβ) can lead to the activation of cardiac fibroblasts [40,41]. Furthermore, mast cell accumulation has been observed at sites of injury [12]. These cells degranulate and release histamine, which stimulates fibroblast proliferation and collagen synthesis [12]. The relative contribution of pro-fibrotic factors inducing fibroblast activation depends on the underlying disease or injury. Activated fibroblasts have an increased proliferation and migration capacity [42]. ECM synthesis and deposition are further characteristics of activated myofibroblasts [43]. To date, the role of fibroblasts in the heart has been highly underresearched. It is known that these cells are important mediators for cardiomyocyte synchronization. In contrast to cardiomyocytes however, cardiac fibroblasts do not generate an action potential and are not excitable, although they are capable of electric coupling amongst each other and to neighboring cardiomyocytes [36]. In vitro, electric signal transduction of cardiac fibroblasts up to a distance of 300 μm has been described [44]. More research is needed in order to understand the role of cardiac fibroblasts in both physiological and pathological heart development, and simply to shed light on the biology of these cells.

5. Diagnostic tools

The current gold standard of diagnosing cardiac fibrosis is the detection and quantification of the interstitial collagen content based on an endomyocardial biopsy [4,45]. Non-invasive techniques exist to identify fibrotic tissue. The most common method for determining LV volume and mass is cardiac magnetic resonance (CMR) imaging. To assess replacement cardiac scar fibrosis, an event that often occurs following MI, gadolinium-based contrast agents are employed, whereby the quantification of the extracellular volume is performed via T1 mapping. This method enables the characterization of the cardiac tissue composition [29]. CMR is however an expensive method requiring considerable skills for acquisition and analysis of the acquired images [29]. Another diagnostic tool is speckle tracking or integrated backscatter echocardiography [5]. With ultrasound natural acoustic reflections, so-called speckles, are detected, which show various characteristic patterns throughout the myocardium [29]. Although quantitative and functional assessment is possible [4], the method depends highly on image quality, which unfortunately varies between operators but also patients [29]. In order to detect perfusion defects or a metabolism perfusion mismatch, nuclear imaging like single photon emission computed tomography (SPECT) or positron emission tomography (PET) have been used respectively [5]. In addition to imaging methods, non-invasive fibrosis detection is also possible utilizing biomarkers. Here, the ratio of matrix metalloproteinase type 1 to tissue inhibitor of metalloproteinase type 1 (MMP-1/TIMP-1) or carboxy-terminal pro-peptide of pro-collagen type I (PCIP) in blood is routinely used [4]. Today, all of the here mentioned methods and techniques are routinely employed. However, none of them fulfills all requirements to identify myocardial fibrosis and therefore a combination of imaging, biomarker assessment and routine histological and histochemical staining is usually needed to fully

characterize cardiac fibrosis [29]. Accordingly, there is a dire need to develop new and most importantly non-invasive methods and technologies for the assessment of fibrosis in vivo.

6. Therapies and therapeutic strategies

Although fibrotic responses include fibroblast recruitment and activation, which potentially lead to scar formation, these events are also crucial for normal wound repair [36,46]. Therefore, it is necessary to understand the details of the events that lead either to physiological or to pathological tissue remodeling in order to design new therapeutic targets or strategies. A strong focus has been on the modification of scar properties, and encouraging the heart to form "better" and more functional scars [36]. Currently used therapeutics to impact the fibrotic response in injuries are angiotensin (AT)-converting enzyme and ATI receptor antagonist, β-blockers, endothelin antagonists, as well as statins [47]. In addition, eplerenone (FDA approved since 2002) has been introduced as a drug suppressing fibrosis formation by blocking the aldosterone pathway [48]. There are also approaches to influence fibroblast activation by blocking TGFβ or Smad3 signaling [49]; however, there is a need for implementing more targeted interventions. There are currently a number of open questions regarding what, when and how to target fibrosis, particularly when blocking certain mediators in a wrong phase can impact cellular responses that are mandatory for cardiac repair [36]. Rog-Zielinska and colleagues nicely reviewed potential targets for a therapeutic manipulation such as cellular recruitment, scar mechanics, cardiomyocyte-fibroblast coupling or targeting microRNA (mRNA)-, periostin- or caveolin-signaling [36].

In order to induce cardiac regeneration, biomaterial-assisted strategies attract more and more attention [50]. It can be distinguished between three biomaterial-based approaches for MI treatment: (i) to introduce a polymeric mechanical support, (ii) the use of an in vitro tissue-engineered cell-containing substrate, or (iii) in situ tissue engineering by delivering active molecules [51]. In order to provide ventricle stability, hydrogels [50] and also polymeric meshes [51] were applied to serve as a mechanical support leading to improved or preserved cardiac function. Another biomaterial-based approach is the classical tissue engineering set up, where cells are seeded onto biomaterial scaffolds prior to implantation. Such cell-containing scaffolds can significantly improve cell survival and enable cell immobilization at the site of injury [50]. Wang et al. employed a thermo-responsive hydrogel with immobilized brown adipose tissue-derived stem cells and demonstrated enhanced cell survival as well as cardiomyocyte differentiation [52]. The third approach of using biomaterials for MI treatment is the immobilization of functional molecules. By encapsulating proteins, growth factors or small molecules in a synthetic or natural biomaterial scaffold, both, structural support and biochemical information can be provided [53–55]. Furthermore, mRNA encapsulation is a promising approach as recently demonstrated in a study, where mRNA-29B was delivered in a collagen type I hydrogel. With this approach, the natural balance of collagen type I: collagen type III ratio was restored [56]. Recently, Lakshamnan and Maulik comprehensively reviewed biomaterial strategies for cardiac repair and cardiac tissue engineering, which was mostly focused on promising preclinical concepts using electrospinning to generate fiber-containing scaffolds or applying injectable hydrogels [15]. There is also a currently ongoing clinical trial (NCT02305602) for the investigation of the impact of a porcine decellularized myocardial matrix hydrogel on the infarcted myocardium [57]. Additionally, a phase I trial using autologous stem-cell sheets as a therapy to treat cardiomyopathy showed promising results regarding safety and functional recovery [58].

Another therapeutic strategy is the application of growth factors and cytokines such as VEGF, IGF-1, HGF, NRG-1, EGF, FGF, and, as already described, TGFβ to induce cardiac repair [24]. With this approach, residing cells in the heart can be targeted and cell processes like survival, migration, proliferation and differentiation can be impacted [24]. Korf-Klingebiel et al. identified myeloide-derived growth factor (MYDGF) as a protecting and repairing protein, which is secreted by macrophages and monocytes after MI [59]. In in vivo MI studies, a significant reduction in scar size was observed after recombinant MYDGF treatment [59]. Furthermore, it has also been suggested to intervene in collagen synthesis by inhibiting pro-collagen N-and C-proteinases enhancer (PCPE) [4]. Other studies demonstrated that the glycoprotein endoglin is upregulated in heart failure. Targeting endoglin to control myocardial fibrosis may be also a promising approach [60].

The application of stem cells has been pursued as another possibility of treating cardiac fibrosis and heart failure. Various phase I and II clinical trials were performed in the last years injecting adult stem cells such as mesenchymal stem cells, cardiac-derived (stem) cells or bone marrow-derived cells [61], but also pluripotent stem cell-derived cells [62]. However, there is a lack of significant benefit by using these cells, presumably due to discrepancies in reporting, variations in trial methods or questionable end points [61]. The modest success of stem cell strategies are according to Mohsin et al. a consequence of poor survival, marginal proliferation and limited engraftment [63]. In addition, it has been demonstrated that the age of cells plays a crucial role in performance [63]. So far, there is no evidence that injected stem cells differentiate towards fully functional cardiomyocytes. Current studies suggest that the partly beneficial effect is a result of secreted paracrine factors, which activate endogenous pathways [61]. According to these findings the question arises, why not to choose the more cost-effective alternative and directly apply these paracrine factors in order to treat the damaged heart or reduce fibrosis. For that, it is necessary to further investigate which factors exactly trigger healing processes.

7. Conclusion

Physiological ECM remodeling after injury is a natural and important process of tissue regeneration. In contrast, pathological remodeling including fibrotic collagen production leads to non-functional scars and an impaired tissue function. Although it is known that different kinds of cardiac fibrosis exist, events that lead to the onset of fibrosis and the process of cardiac scar formation are still not entirely understood. Deciphering the biology that underlies these events will enable the design of new, safe and effective treatment strategies. In addition, there is also a need of improving diagnostic tools in order to enable early, reliable and non-invasive fibrosis detection, which will allow to improve the patients' quality of life.

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