Review Article

Cardiac fibrosis in regenerative medicine: destroy to rebuild

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Abstract: The major limitations for cardiac regeneration in patients after myocardial infarction (MI) are the wide loss of cardiomyocytes and the adverse structural alterations of extracellular matrix (ECM). Cardiac fibroblast differentiation into myofibroblasts (MFB) leads to a huge deposition of ECM and to the subsequent loss of ventricular structural integrity. All these molecular events depict the fundamental features at the basis of the post-MI fibrosis and deserve in depth cellular and molecular studies to fill the gap in the clinical practice. Indeed, to date, there are no effective therapeutic approaches to limit the post-MI massive fibrosis development. In this review we describe the involvement of integrins and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)/ADAMTS-like (ADAMTSL) proteins in cardiac reparative pro-fibrotic response after MI, proposing some of them as novel potential pharmacological tools.

Keywords: Cardiac fibrosis; myocardial infarction (MI); integrins; a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS); cardiac regeneration

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Introduction

During the last 10 years, cardiac fibrosis has assumed a central role in the cardiovascular field and could be defined as one of the rising topic in the cardiac research. The quantification of scientific papers (original manuscript and reviews) focused on 'cardiac fibrosis' present on PubMed database lends credence and veracity to the statement above. The bar graph of *Figure 1* indicates the number of publication centred on fibrosis in the heart, which is interestingly almost tripled in the last decade.

Myocardial infarction (MI), together with other detrimental stimuli (i.e., pressure overload), is the starting point of all the adverse structural alterations of extracellular matrix (ECM) occurring in the left ventricular (LV) remodelling (1,2). Since LV pathological changes are due to the post-MI reparative processes (i.e., inflammation, cardiac fibrosis), the study of these events by a cellular and molecular point of view is fundamental for its impact on clinical practice. In fact, it is extremely important to counteract and limit the infarcted area expansion and, to do this, physician routinely perform coronary artery reperfusion (3). Nonetheless a specific therapeutic approach blocking inflammation and the subsequent fibrotic reparative process is still missing (4,5).

The crescent knowledge on the dynamic features of myocardial ECM has unveiled new roads for limiting myocardial fibrosis in terms of type I collagen accumulation and its severe clinical consequences, such as heart failure (HF) (6-9). Interestingly, beyond the structural proteins, ECM contains also non-structural compounds with regulatory roles, such as proteases, which foster changing in collagen structure and content in a rapid temporal fashion (10). Moreover, recent studies have suggested a plethora of

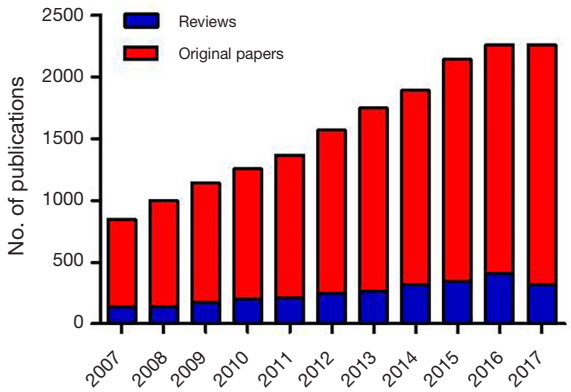

Figure 1 Scientific production of the last 10 years in the cardiac fibrosis field. Number of original manuscript and review articles spanning from 2007 to 2017 found in PubMed database (https://www.ncbi.nlm.nih.gov/pubmed) searching the string "cardiac fibrosis".

potential therapeutic targets that may influence cardiac wound healing and repair (11).

This review is aimed to consider cellular and molecular mediators involved in post-MI repair, pointing out the attention on two protein families that could potentially play a key role in the amelioration of adverse remodelling and cardiac fibrosis development.

Cardiac ventricular fibrosis

Although the process of cardiac fibrosis could be considered an adaptive and protective mechanism, over the time it progresses in an uncontrolled way, driving irreversible remodelling and determining significant impairment in heart function (6).

The ventricular remodelling is a dynamic and complex process resulting from activation of cellular events and molecular pathways which involve several cardiac cell types such as cardiomyocytes, fibroblasts, vascular and immune cells. In pathological conditions, clinical ventricular remodelling may depict three major patterns: (I) a concentric remodelling, when pressure overload determines cardiomyocyte thickening and ECM protein deposition; (II) an eccentric remodelling, resulting from a volume overload that produces cardiomyocyte lengthening; and (III) a post-MI remodelling, which is determined by a combined pressure and volume overload on the non-infarcted area (12). In this review we point out on post-MI remodelling, which occurs after MI damage subsequent to cardiomyocyte necrosis and leads to a wound healing process named reparative fibrosis (13,14). From a molecular point of view, in this type of fibrosis the new deposed ECM, particularly reach in collagen fibres, takes the place of necrotic cells.

A huge number of studies, well reviewed by Prabhu *et al.* in 2016, have shown the important role played by several molecular and cellular inflammatory mediators in the establishment of the fibrotic process (4). In depth, cardiac repair after MI results from a series of events which begin with an initial phase of sterile inflammation and immune cell infiltration, also called inflammatory phase. This fundamental step leads to digest and clear damaged cells and ECM. A large number of well-known danger-associated molecular patterns is involved in this specific step. Among them, HMGB1, S100 proteins, the extra domain A of fibronectin (ED-A FN), several cytokines and chemokines, such as interleukin (IL)-1α, IL-6, TNF-α are noteworthy (15-18). After that, a reparative phase occurs determining the resolution of the inflammation state, the proliferation of fibroblasts and their differentiation into myofibroblasts (MFB), scar formation and neovascularization (19,20). Although inflammation plays a fundamental role in the progression of post-MI ventricular remodelling, to date is not available a selective therapeutic tool able to effectively turn it off.

Despite the initial trigger leading to fibrous tissue depends on different types of stimulus, there are several molecules leading to increased production of pro-fibrotic mediators, such as the anti-inflammatory IL-10 and the transforming growth factor-β (TGF-β), which acts locally as "master switch" from inflammation to reparative process (12,21). In depth, TGF-β is a pleiotropic cytokine, critically regulating a wide variety of cell functions like growth, proliferation, differentiation, but also ECM deposition. Three structurally similar isoforms of TGF-β (TGF-β1, 2 and 3), encoded by three distinct genes, have been identified in mammalian species (22). TGF-β1 is the prevalent isoform and it has been found almost ubiquitously, whereas the other isoforms are expressed in a more limited spectrum of cells and tissues (21). TGF-β is produced by many cell types and is secreted as a latent small complex (LSC) composed by C-terminal mature TGF-β and its N-terminal pro-domain, the latency-associated peptide (LAP) (23). This complex is further linked to the latent TGF-β binding proteins (LTBP), an ECM fibrillin-like protein family, to form the large latency complex (LLC). Once secreted, LLC is covalently cross-linked to the ECM proteins by the activity of extracellular tissue

transglutaminase (24-26). In this conformation, TGF-β is unable to associate with its receptor, so its activation is primarily regulated by its release from the LLC.

Over the past several years, it has been demonstrated that stimuli which can induce protein denaturation (e.g., acid or alkaline pH in extracellular milieu, brief increases in temperature and exposure to oxidants) or proteolysis by the activity of proteases, thrombospondin-1, matrix-metalloproteinase (MMP)-2, and MMP-9 determine the release, and so the activation of TGF-β (27-29). The released TGF-β is able to bind the constitutively active TGF-β type IIreceptor (TβRII). Then, the ligand-receptor complex recruits the type I receptor of TGF-β (TβRI), also known as ALK5, which is expressed by many different cell types. In endothelial cells there is a second TβRI, named ALK1. The activation of both TβRI types, due to their trans-phosphorylation, propagates downstream intracellular signals through the SMAD proteins. While SMAD2 and SMAD3 are activated by ALK5 phosphorylation, SMAD1, SMAD5 and SMAD8 are all activated by ALK1 (30).

To date, it is well-known the key role of TGF–β1 in mediating cardiac hypertrophy (31) by stimulating (I) cardiomyocytes hypertrophy; (II) fibroblast activation and proliferation; and (III) ECM protein synthesis (i.e., collagen) in cardiac tissue (12,32). Notably, the activation of a small fraction of latent TGF-β1 is sufficient to generate maximal cellular response (27). *In vitro* and *in vivo* studies with models lacking TGF-β1 strongly contributed to highlight its involvement in several cell functions and, in the meantime, unveiled its pleiotropic role and the great complexity of its management. In this context, gene therapy experiments conducted on a MI model by local transfection of TβRII extracellular domain suggested that early inhibition of TGF-β1 may exacerbate cardiac dysfunctions, while late neutralization of TGF-β signalling may protect from interstitial fibrosis and hypertrophic remodelling (33). Moreover, it has been observed that inhibition of TGF-β after MI resulted in an early mortality caused by cardiac rupture, whereas cardiomyocyte-specific suppression of both TβRI and TβRII stimulated anti-inflammatory and cytoprotective responses (34). Thus, the detrimental effects of early TGF-β inhibition after MI may not lead to a direct action on cardiomyocyte survival, but may determine a loss of anti-inflammatory function on all cardiac cell types (e.g., inflammatory cells, endothelial cells, fibroblasts).

One of the key function of TGF-β1 is the well-known phenotype switch of fibroblast into MFB (35). Morphologically, MFB are characterized by the presence of a contractile apparatus composed by bundles of α-SMA microfilaments and contractile proteins. This apparatus provides a mechano-transduction system able to generate forces by stress fibres, that can be transmitted and transduced by the surrounding ECM into intracellular signals (36-40). To further produce the tension necessary to activate this mechano-transduction, ECM production by MFB is enhanced in the process of remodelling. The most prominent MFB-derived ECM products are type I, III, IV, V, and VI collagen (41). However, the most reliable marker of MFB-derived ECM is the ED-A FN (42) which is also expressed in low amounts by cultured fibroblasts (36,43) and by vascular smooth muscle cells, both *in vivo* and *in vitro* (44). Recently, type VI collagen attracted attention as it is up-regulated during myocardial interstitial fibrosis (45) as well as during the fibrotic process in other tissues. It is important to point out that MFB are not present in healthy myocardium, but they are detectable in this region following cardiac injury (46). The origin of MFB in the infarcted area may be ascribed to resident fibroblasts (47) and/or circulating bone marrow progenitors (48), but this remains a debated issue. Precisely, interstitial fibroblasts that survive to ischemic insult and/or cells recruited from neighbouring viable areas may undergo MFB differentiation in response to increased levels of bioactive TGF-β and the subsequent changes in ECM composition. Additional sources of MFB in the healing infarcted area may be represented by endothelial-to-mesenchymal transition of endothelial cells (49), epicardial epithelial cells (50), as well as pericytes (51). Moreover, marked induction of chemokines in response to extensive cardiomyocyte necrosis may result in recruitment and activation of additional subsets of reparative fibroblasts that play an important role in scar formation (4).

Arising targets to limit fibrosis

The amelioration of cell engraftment in cell therapy after MI, to date, is one of the fundamental issue still open in the field of cardiac regenerative medicine. It is noteworthy to mention this matter, since fibrosis plays one of the major guilty role in the incorrect engraftment and subsequent scanty survival of injected cells to treat the impaired cardiac tissue (52,53). In fact, the pathological remodelling of the heart undergoing chronic fibrosis is a significant cause of mortality in cardiovascular disease and there are still no available therapies to reverse or limit its effects (6). Thus, researchers are evaluating novel targets towards the intricate pattern of pathological fibrosis to

discover potential therapies in the context of cardiac regeneration. Among the several proteins involved as key participants in the cardiac fibrotic pathways, recently both the integrins and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteins have been put in the spotlight. These protein families are strongly related to the TGF-β signalling and involved in the ECM remodelling process which is not only linked to post-MI context, but also to other pathological scenarios (54-57). In particular, the integrins are transmembrane glycoproteins connecting cells with ECM proteins and modulating tissue homeostasis and architecture. The ADAMTS superfamily comprises extracellular enzymes involved in different pathways including maturation of pro-collagen and pathophysiological tissue remodelling (55).

In this review we particularly focus on those proteins belonging to the integrin and ADAMTS families that are expressed in the heart and have the potentiality to counteract the cardiac pathological fibrotic process.

The integrins

The integrins literally constitute the bridge between cells and ECM proteins maintaining a sophisticated structure able to anchor cells and sustain tissue functions in physiological conditions. Nonetheless, integrins are strongly involved in the pathological context of several tissue impairments (58). Integrins are a large family of glycoproteins and act both as adhesive receptors and intracellular signalling events mediators. The integrins are heterodimeric receptors composed by a dimer of an unrelated α and β subunits (59). Currently, in mammalian cells there are 18 α and 8 β known subunits which are able to form at least 24 distinct combination of integrin heterodimers (60). Each subunit contains a large extracellular domain (700–1,100 amino acids), a transmembrane α-helix domain and a short cytoplasmatic tails ranging from 20 to 60 amino acids (61). The integrin binding to ECM glycoproteins, including collagens, fibronectins, laminins, and cellular receptors (i.e., VCAM-1 and ICAMs), is strongly dependent to the presence of divalent cations, typically Ca^{2+} and Mg^{2+} (60,62). Concerning molecular functions, integrins participate to a wide range of biological events, including organogenesis, cell-cell and cell-ECM attachment and transduction of signals involved in cell proliferation, differentiation, migration and death. In the cardiovascular system, integrins are expressed in cells of vasculature, blood as well as neurons, cardiac myocytes, and non-muscle cardiac cells. Few studies have shown that integrins are involved in heart formation (63) and function, but also in the development of cardiac diseases (64).

Interestingly, the transcriptional control exerted by TGF-β can strongly affect integrin-mediated processes basically through its regulatory activity on the expression of integrin ligands (i.e., tenascin, vitronectin, fibronectin, members of the laminin and collagen families) and on the stimulation of some integrin-associated protein expression. In 2004, Keski-Oja *et al.* proposed a non-proteolytic mechanism of TGF-β activation, named "traction model" since it occurs through cell traction forces exerted by the actin cytoskeleton. These forces are translated by integrin into a conformational change of the LLC complex leading to the exposure, and the consequent activation, of TGF-β (65-68). Of note, the LAP of TGF-β1 and TGF-β3, but not TGF-β2, contain the arginine-glycine-aspartate (RGD) motif which can be bound by the αv-containing integrins, αIIbβ3, α5β1 and α8β1 (56). Interestingly, non-proteolytic activation of latent TGF-β has been demonstrated *in vitro* for αvβ3, αvβ5 and αvβ6, as well as for β1-containing integrins with a still unidentified α-subunit (68). However, the physiological relevance of the TGF-β activation by β1-containing integrins still remains controversial.

Among this large protein family, αv integrins form a subgroup of five members (αvβ1, αvβ3, αvβ5, αvβ6, and αvβ8) all able to recognize a group of overlapping ligands which generally contain the canonical RGD motif (69). These integrins are widely expressed on multiple cell types and, during development, the different αv-associated β subunits show distinct expression patterns (70-72) that, together with the wide range of potential ligands, imply several functions for different receptors. Significant expression of αv integrins has been noted in particular cell types, such as neural crest (73), glial, muscles (70,74,75), and epithelial cells (76,77) as well as osteoclasts (78), blood vessels during development (79-83), and cardiac fibroblasts (84).

Inactivation of αv integrins by $\alpha v^{-/-}$ mice models yielded the death during embryonic development or soon after birth as a result of intracranial and/or gastrointestinal hemorrhage (85). Although the limited survival of mice lacking all αv integrins, the availability of live mice lacking a single αv integrin has made possible a series of studies identifying a number of previously unexpected *in vivo* roles for these proteins. On the basis of the studies involving knockout mice and/or the use of specific integrin antagonists, are emerging novel insights of this integrin subfamily, which play important and specific roles in (I)

determining growth and permeability of blood vessels; (II) regulating tissue inflammation and fibrosis; and (III) developing several common lung diseases (86). Importantly, the αv integrin-mediated activation of TGF-β has been highlighted *in vivo* by mutation of the RGD site of LAP leading to defects observed in TGF-β1-null mice (87). In addition, genetic ablation of the β6-subunit, or conditional deletion of αv or β8 from dendritic cells, causes exaggerated inflammation as a result of impaired TGF-β signalling (88,89). The phenotype of mice lacking both the αvβ6 and αvβ8 integrins recapitulates the abnormalities observed in TGF-β1 and TGF-β3, but not in TGF-β2, knockout mice, indicating that the integrins αvβ6 and αvβ8 can account for the full activation of TGF-β1 and TGF-β3 *in vivo*.

The first clue that the integrin-TGF-β interplay is central in fibrosis became from observation on mice lacking the β6-subunit, which are partially or completely protected pulmonary fibrosis induced by radiation or bleomycin (90,91). In addition, low doses of antibodies against αvβ6 prevent radiation- or bleomycin-induced pulmonary fibrosis in mice, without causing inflammation (92,93). Furthermore, it has been shown that constitutive expression of αvβ6 in the basal layer of the epidermis leads to elevated TGF-β1 activation and the development of spontaneous chronic ulcers with severe fibrosis (94). In wild-type mice, fibrosis can be equally inhibited by treatment with antagonists of TGF-β signalling or by using a blocking antibody against αvβ6 (95,96). The αvβ6 integrin is not normally expressed in healthy epithelia, but its expression is induced in several human fibrotic disorders involving kidney, liver and lung in terms of sclerosis and idiopathic pulmonary fibrosis. Specifically, the inhibition of αvβ6-induced TGF-β activation at sites of injury is a promising therapeutic tool to prevent TGF-β-mediated fibrosis.

Mice lacking β3 and/or β5 integrins do not develop abnormalities similar to those caused by TGF-β signalling deficiency (97-99). Nevertheless, αvβ3- and/or αvβ5-mediated TGF-β activation has been reported as an important clue in pathological conditions. In fact, increased expression levels of both these integrins have been observed in dermis of patients with scleroderma, a chronic disease involving cutaneous manifestations of fibrosis. In this pathological context, these integrins elicit *in vitro* autocrine TGF-β signalling in patient-derived fibroblasts (100-103). The TGF-β activation by αvβ5 is important also in pulmonary fibrosis, whereas the contribution of αvβ3 in this human pathology has not been yet established. In human fibrotic lungs, epithelial cells expressing αvβ5 and the protease activated receptor 1 co-localize with MFB, and TGF-β-mediated pulmonary fibrosis is reduced by the blockade of αvβ5 in a mouse model (104).

Astoundingly, Henderson *et al.* reported that αv-containing integrins on MFB are components of a core cellular and molecular pathway contributing to pathological fibrosis in multiple solid organs and suggested that the targeting of this pathway could lead clinical utility in the treatment of patients with a broad range of fibrotic diseases (105).

As previously mentioned, MFB are not present in healthy adult myocardium and appear after cardiac injury (106). It has been also reported that MFB result from the differentiation of resident cardiac fibroblasts (107) or from the trans-differentiation of endothelial cells (49,108). The endothelial-to-mesenchymal transition can be induced by TGF-β in a SMAD-dependent fashion during cardiac fibrosis, while BMP-7 is able to block this process acting as an anti-fibrotic factor (49).

Data from preclinical models suggest that integrin-mediated TGF-β activation is involved in several pathological conditions, such as scleroderma, lung, kidney and liver fibrosis (87). In particular, *in vitro* studies demonstrated that both integrin αvβ6 and αvβ8-mediated TGF-β activation play a key role in lung fibrosis. Indeed, the first one promotes squamous metaplasia of airway epithelial cells (109) while the second inhibits airway epithelial proliferation and migration (110). These results were confirmed by using β6 integrin subunit-null mouse model and airway fibroblast isolated from patients with chronic obstructive pulmonary disease (92). Moreover, it has been shown that the expression of αvβ6 is increased in the migrating epidermal cells adjacent to wound (111), suggesting that this integrin is also involved in skin fibrosis, as confirm by *in vivo* studies using αvβ6-deficient aged mice (94,112). In addition, several evidences reported that also αvβ3 and αvβ5 integrins, which are able to activate TGF-β in primary culture isolated from scleroderma fibroblasts, are increased in the dermal fibroblasts of biopsy sample from scleroderma patients (100,101,103). Furthermore, Hahm *et al.* demonstrated that αvβ6 enhances renal fibrosis by using $Col4a3^{-/-}$ mice deficient in αvβ6 integrin or, alternatively, $Col4a3^{-/-}$ mice treated with anti-β6 integrin-blocking antibodies (or a soluble TβRII) (95).

Taken this large amount of data together, it is clear that all these features render nowadays the integrins one of the most interesting therapeutic target on which investigate in the field of specific fibrotic disorders (113). Proofs of this sentence may be appreciated by the large literature,

ultimately and notably reviewed in this therapeutic context by Hatley and colleagues in 2017 (114). Although the integrin family was initially identified to have a key role in mediating cell adhesion, it is becoming even more clear that a subset of integrin plays the role of the culprit along with TGF-β in the fibrotic process.

The ADAMTS proteins

The ADAMTS proteins are members of a superfamily that includes 19 metalloproteases and a subfamily composed by 7 ADAMTS lacking their catalytic activity, called ADAMTS-like (ADAMTSL) proteins (55). While the ADAMTS are involved in different pathways, including maturation of pro-collagen and tissue remodelling in several pathophysiological conditions (i.e., angiogenesis, arthritis), the ADAMTSL are component of the ECM with functions potentially linked to the regulation of ADAMTS protein activity (57).

In the cardiovascular context, the role of ADAMTS proteins is arousing growing interest since some of them have been recently found in the culprit plaques of patients with MI and others show versican cleaving activity (6,115-117). Specifically, an up-regulation of ADAMTS2, ADAMTS3, and ADAMTS13 have been observed in coronary lesions of patients with MI highlighting their possible participation in cardiovascular disease (116). Interestingly, both the ADAMTS2 and the ADAMTS3 have pro-collagen N-propeptidase activity, while the ADAMTS13 is a von Willebrand factor cleaving protease. ADAMTS2 is expressed in several tissues, in addition to the heart, and it is involved in multiple processes. Among them, ADAMTS2 primary function is to activate the types I, II, III and V pro-collagen by the promotion of their cleavage (118). Mutations in ADAMTS2 determine enzyme malfunction and cause the Ehlers-Danlos syndrome type VIIC, a rare connective tissue disorder determined by the failure of type I pro-collagen cleavage (119). Dong *et al.* reported in the fibrotic pathological context of hepatic cirrhosis the pro-fibrotic role of ADAMTS2 where resulted highly expressed and positively correlated with TGF-β levels. In turn, TGF-β probably induces ADAMTS2 expression through the SMAD signalling (120). Conversely, Wang *et al.* recently evidenced a protective role for ADAMTS2 in rat angiotensin II-dependent cardiomyocyte hypertrophy and in a murine model of cardiac hypertrophy induced by pressure overload. Moreover, they also demonstrated in patients with dilated cardiomyopathy that cardiac hypertrophy is hampered by ADAMTS2 expression levels (118). Indeed, under pathological conditions ADAMTS2 is able to protect the heart preventing hypertrophy by the inactivation of the PI3K/AKT signalling pathway, a recognized key mediator of cardiac hypertrophy. For this role in modulating cardiac fibrosis and its easy and effective manipulation as extracellular protein, ADAMTS2 has been proposed as novel pharmacological tool (118).

Among the three proteins found up-regulated in the coronary lesions of patients with MI, ADAMTS3 plays the most complex role due to its involvement in several biological processes not always related to collagen maturation (i.e., blood coagulation, neoangiogenesis, development, male fertility). To our knowledge its role in cardiovascular fibrosis have not been already clarified (121).

Although ADAMTS13 is principally related to the development of thrombotic thrombocytopenic purpura—a rare disease characterized by thrombocytopenia, hemolytic anemia, and thrombi formation in the microvasculature—there is increasing evidence that its malfunctioning has a role in adverse cardiovascular events, including HF (122-127). Interestingly, the peculiar von Willebrand factor cleavage activity of ADAMTS13 has been recently exploit to investigate its effect on chronic myocardial injury in a pressure overload mouse model. Specifically, the recombinant human ADAMTS13 administration determined *in vivo* a strong improvement in myocardial remodelling and functionality due to the ADAMTS13 action in preventing inflammation, platelet recruitment and microvessel obstruction (128). Of note, the recombinant human ADAMTS13 is currently used in a clinical trial for the treatment of thrombotic thrombocytopenic purpura, so it may be exploitable as a new therapeutic tool against fibrotic cardiac damage (129).

Another interesting function attractive for researchers regards ADAMTS1 and ADAMTS4 which are capable to cleave versican, the primary proteoglycan component of the vasculature. In the cardiovascular context, ADAMTS1 and ADAMTS4 ability in ECM rearrangement results crucially detrimental both for the ventricular remodelling after MI and the regulation of fibrous cap stability in atherosclerotic plaque (117,130). The damaging role of ADAMTS1 is basically due to its cleavage activity of versican that, once cleaved, stimulates vascular smooth muscle cell migrations (131). Besides, ADAMTS1 has many beneficial functions including anti-angiogenic activity, versican turnover during mouse cardiac development, and type I collagen degrading activity (57,132-135). Interestingly, the ability

of ADAMTS1 to degrade type I collagen has been recently demonstrated in a mouse model of chronic viral myocarditis (CVMC), a disease that, at chronic stage, is characterized by ECM accumulation in heart tissue contributing to cardiac function loss (115). In this context, ADAMTS1 myocardial protein expression has been found to be inversely correlated with the expression levels of type I collagen, and positively correlated with the carboxyterminal telopeptide of type I collagen, a degradation marker of type I collagen, released during its breakdown (115). Furthermore, *in vitro* studies provided evidence that, during the progression of CVMC to dilated cardiomyopathy, IL-17 as well as TGF-β are able to up-regulate ADAMTS1 expression, increasing type I collagen degradation (136). The same function of ADAMTS1 has been demonstrated in murine CVMC model after treatment with the angiotensin II converting enzyme inhibitor (ACEi) captopril. In this scenario ADAMTS1 contributes to the anti-fibrotic effect of captopril by accelerating type I collagen degradation (115). Although nowadays the exact underlying mechanism remained to be determined, the link between ACEi and ADAMTS1 opens new perspectives for potential combined therapy (115,137).

Concerning ADAMTS4, it has a fundamental role in degradation versican and aggrecan and thus it is counted among the pro-fibrotic ADAMTS proteins (117,138). This is basically because a link between the ADAMTS4 activity and HF development has been described (138). In depth, in the myocardium of rats subjected to pressure overload by aortic banding was observed an increased ADAMTS4 versicanase activity. Furthermore, the inhibition of ADAMTS4 expression and activity by pentosan polysulfate (PPS) treatment improved cardiac contractile performance (138). On the basis of these discoveries together with the proven positive effect of PPS in reducing the infarct size in reperfusion models, the inhibition of ADAMTS4 is now depicted as a promising novel therapeutic approach in HF. Of note, the modulation of ADAMTS4 activity could be of great importance also in the atherosclerotic context where TGF-β-dependent inhibition of ADAMTS4 secretion by macrophages contributes to plaque stabilization (117).

Another member of the ADAMTS family linked to TGF-β signalling is ADAMTS5 which is principally involved in cartilage aggrecanase activity and joint fibrosis (139,140). In detail, ADAMTS5 determines *in vivo* the balance of proteoglycan turnover in the derma and, when absent, the TGF-β signalling intensification (140). In murine model of atherosclerosis ADAMTS5 promotes also lipoprotein retention. It has been properly found that ADAMTS5 determines the physiological release of versican and aggrecan fragments, and that its reduction is accompanied by the accumulation of biglycan and versican, the major LDL-binding species (141).

Interestingly, a study using balloon-injured arteries of rats showed that ADAMTS7 determines vascular smooth muscle cell migration and neointima formation probably through the degradation of thrombospondin-5, while another study in mouse model indicated the thrombospondin-1 as principal mediator of neointima formation, determining a retarded re-endothelialization (142,143). Furthermore, it has been also showed that ADAMTS7 accumulates in the smooth muscle cells of coronary and carotid atherosclerotic plaques (144). A new link between ADAMTS7 and the cardiovascular context has been recently drawn by the association between its plasmatic levels and the worsening of LV function in patients with MI. Precisely, in a prospective study conducted by Wu *et al.* on STEMI, non-STEMI patients and controls it has been found that ADAMTS7 levels were greater in patients with LV ejection fraction ≤35%, independently from the STEMI or non-STEMI diagnosis (145). Despite all these results elect ADAMTS7 as a new target for possible post injury vascular intima hyperplasia treatments, further studies are needed to verify its exact mechanism of action in LV remodelling.

Among the ADAMTS family there are also 'sister proteins' involved in the same pathways, but determining opposite effects. This is the case of ADAMTS10 and ADAMTS6, both involved in fibrillin-1 microfibril formation, but with opposite functions. ADAMTS10 is required for focal adhesions, epithelial cell-cell junction formation, and microfibril deposition and it is known to cause, when mutated, the Weill-Marchesani syndrome, while ADAMTS6 has an inhibitory effect on the same pathways (146).

The previously mentioned ADAMTSL proteins are integral components of the ECM and some of them, such as ADAMTSL2, ADAMTSL6 and ADAMTSL4, are interestingly involved in fibrosis. Specifically, ADAMTSL2, when mutated, is the cause of geleophysic dysplasia, an autosomal rare disorder mainly characterized by cardiac valvular thickening and progressive HF leading to premature death. From a molecular point of view, this pathology is characterized by an enhanced ECM mechanical stability (147). In particular, ADAMTSL2 binds both fibrillin-1 and the LTBP-1 determining the increase in the TGF-β activity levels typical of this pathology.

Moving from the cardiovascular to a general context, ADAMTSL6 was found functionally involved in the

organization of the ECM in mice due to its capacity to directly bind fibrillin-1 and to promote its matrix assembly. Interestingly, ADAMTSL6 is highly expressed in murine heart tissue and its direct binding to fibrillin-1 has been demonstrated by surface plasmon resonance binding assay. Moreover, ADAMTSL6 overexpression in transgenic mice determined an excessive fibrillin-1 microfibril formation (148).

For another member of the subfamily, ADAMTSL4, has been described a role strictly related to fibrillin-1 assembly and function in patients affected by ectopia lentis (149). In particular, the dislocation of the ocular lens in these subjects is determined by failed maintenance of the lens in the correct position because of the laxity of their suspensory ligaments principally composed by fibrillin-1. Of interest, mutations in several ADAMTS/ADAMTSL proteins affect the regulation of microfibrils in terms of assembly, stability and anchorage, resembling the phenotype observed in some fibrillin-1 related genetic disorders like geleophysic dysplasia, the Weill-Marchesani and Marfan syndrome, further confirming their functional link with microfibril network (6,150,151).

In conclusion, since several studies on ADAMTS/ADAMTSL proteins provided evidence of their involvement in cardiac fibrosis they may serve as promising targets to be boosted (i.e., ADAMTS2, ADAMTS13) or inhibited (i.e., ADAMTS4) on the basis of their main role for preventing cardiac hypertrophy and HF.

Conclusions

To date, the knowledge of all molecular mediators involved in heart injury, repair, and remodelling after MI has unveiled a series of new possible therapeutic targets supporting cell therapy for patients. In fact, because of the fibrosis, the post-MI cardiac milieu is a discomfort zone to receive and embrace protective and potentially reparative cells. Pharmacological studies are now needed to define the exact effect of novel tools on pathological alterations leading to adverse remodelling after MI. Strategies able to modulate the fibrotic process are not only necessary to reduce, or even avoid, cardiac remodelling and subsequent HF, but may be also crucial in the future obtainment of effective cell-based myocardial regeneration.

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Footnote

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