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Transforming Growth Factor (TGF)-β signaling in cardiac remodeling

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Abstract

Myocardial TGF-β expression is upregulated in experimental models of myocardial infarction and cardiac hypertrophy, and in patients with dilated or hypertrophic cardiomyopathy. Through its effects on cardiomyocytes, mesenchymal and immune cells, TGF-β plays an important role in the pathogenesis of cardiac remodeling and fibrosis. TGF-β overexpression in the mouse heart is associated with fibrosis and hypertrophy. Endogenous TGF-β plays an important role in the pathogenesis of cardiac fibrotic and hypertrophic remodeling, and modulates matrix metabolism in the pressure-overloaded heart. In the infarcted heart, TGF-β deactivates inflammatory macrophages, while promoting myofibroblast transdifferentiation and matrix synthesis through Smad3-dependent pathways. Thus, TGF-β may serve as the "master switch" for the transition of the infarct from the inflammatory phase to formation of the scar. Because of its crucial role in cardiac remodeling, the TGF-β system may be a promising therapeutic target for patients with heart failure. However, efforts to translate these concepts into therapeutic strategies, in order to prevent cardiac hypertrophy and fibrosis, are hampered by the complex, pleiotropic and diverse effects of TGF-β signaling, by concerns regarding deleterious actions of TGF-β inhibition and by the possibility of limited benefit in patients receiving optimal treatment with ACE inhibitors and β-adrenergic blockers. Dissection of the pathways responsible for specific TGF-β-mediated actions and understanding of cell-specific actions of TGF-β are needed to design optimal therapeutic strategies.

Keywords

TGF-β; remodeling; fibrosis; Smad; hypertrophy; angiotensin

Introduction: The biology of TGF-β

The TGF-βs are pleiotropic cytokines, which are implicated in a wide variety of cell functions, critically regulating inflammation, extracellular matrix deposition, cell proliferation, differentiation and growth. Three structurally similar isoforms of TGF-β (TGF-β1, 2 and 3), encoded by three distinct genes, have been identified in mammalian species [1]. TGF-β1 is the prevalent isoform and is found almost ubiquitously, whereas the

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other isoforms are expressed in a more limited spectrum of cells and tissues. Although the three isoforms have similar i*n vitro* properties, their *in vivo* effects are distinct.

TGF–β is produced by many cell types and is secreted as a latent complex, unable to associate with its receptors. Activation of TGF–β signaling pathways is primarily regulated by release of active TGF–β from the latent complex that is stored in significant amounts in most tissues. Activation of a small fraction of latent TGF–β is capable of generating maximal cellular response [2]. The latent dimeric complex contains the C-terminal mature TGF–β and its N-terminal pro-domain, LAP (TGF–β latency-associated peptide) [3] that prevents TGF-β from interacting with its receptors, functioning as an inhibitor due a non-covalent high affinity association with TGF-β Release of bioactive TGF–β requires proteolytic cleavage and separation of LAP from TGF–β a step that involves processing of the proTGF–β complex by a plasma membrane-bound furin, or another extracellular protease, such as plasmin [4]. Once processing has occurred the complex is competent and can be activated. Liberation of active TGF–β from the activation-competent LAP:TGF–β is a poorly-understood process that involves the inducible matricellular protein Thrombospondin (TSP)-1 [5]. Binding of TSP-1 to the sequence LSKL in the LAP alters the conformation of TGF-β making it accessible to its receptor. A variety of other molecules have been described as TGF-β activators. Proteases including plasmin, Matrix Metalloproteinase (MMP)-2 and MMP-9 are capable of activating TGF-β, coupling matrix degradation with activation of a molecule that has a primary role in maintaining matrix integrity and stability [2], [6], [7]. Moreover, reactive oxygen species [8], matricellular proteins [9] and integrin-mediated interactions [10] are also capable of inducing TGF-β activation; their exact role may be dependent on the cell types involved and on the pathologic context.

TGF–β signals through the constitutively active type II receptor (TβRII) at the cell surface (Figure 1). The complex subsequently recruits and transphosphorylates the type I receptor (TβRI), also known as ALK5 [11]. Apart from the well-characterized ALK5, which is expressed by many different cell types, endothelial cells express a second type I TGF–β receptor, termed ALK1 [12]. TβRI activation propagates downstream intracellular signals through the Smad proteins [13]; Smad2 and Smad3 are activated through phosphorylation by ALK5, whereas Smad1, Smad5 and Smad8 are activated by ALK1 as well as the Bone Morphogenetic Protein (BMP) receptors [14]. These receptor-activated Smads (R-Smads) form complexes with the common Smad, Smad4, and translocate to the nucleus, where they activate or repress gene transcription depending on the recruitment of coactivators or corepressors into transcriptional complexes [15]. The structurally divergent Smad6 and Smad7 inhibit TGF–β signaling (inhibitory Smads) by interfering with R-Smad activation. Smads1 and 5 induce Smad6 expression, whereas Smad3 induces Smad7 expression triggering an inhibitory feedback loop that suppresses TGF-β-mediated effects. Besides Smad-mediated transcription, TGF-β activates many non-canonical signaling pathways including Erk, JNK, p38 MAPK and small GTPase pathways [16] [17], [18].

Cellular effects of TGF-β

The TGF-βs are some of the most pleiotropic and multifunctional peptides known. They exert potent and diverse effects on many different cell types and are involved in a wide variety of biological processes such as embryonic development, cell growth and differentiation, cell proliferation and survival, fibrosis and regulation of the immune and inflammatory response. The actions of TGF-β on a specific cell type are affected by its state of differentiation and by the cytokine milieu [19]. Mice with genetic disruptions of the TGF-β genes have revealed essential, but distinct roles for all TGF-β isoforms in embryonic development and immune regulation. Approximately 50% of TGF-β1 −/− mice die *in utero*

due to defective yolk sac vasculogenesis and hematopoiesis [20]; the remaining animals show no gross developmental abnormalities, but about 2–4 weeks after birth they succumb to a wasting syndrome due to multifocal inflammation in many organs, but primarily in the heart and lungs [21]. TGF-β2 knockouts exhibit perinatal mortality and a wide range of developmental defects [22]. TGF-β3 deficient animals exhibit defective epithelial-mesenchymal interactions resulting in cleft palate and abnormal lung development [23]. The distinct phenotypes of the TGF-β null animals indicate distinct non-compensated functions of the three TGF-β isoforms.

Beyond its homeostatic role, TGF-β regulates phenotype and function of all cells involved in tissue injury, repair and remodeling (Figure 2). The effects of TGF-β on inflammatory leukocytes can be either stimulatory or inhibitory, depending on the cytokine milieu and their state of differentiation [24], highlighting the pleiotropic nature of the cytokine. TGF-β inhibits IL-2-dependent T lymphocyte proliferation [25], is an important negative regulator of B-lymphopoiesis [26], and induces regulatory T cell differentiation [27]. Femtomolar concentrations of TGF-β induce monocyte chemotaxis [28]; picomolar concentrations activate monocytes, stimulating synthesis of cytokines, chemokines and growth factors [19], [28] and increasing integrin expression. In contrast to the activating effects of TGF-β on peripheral blood monocytes, its actions on mature macrophages are predominantly suppressive, leading to markedly reduced cytokine and chemokine synthesis [29] and decreasing reactive oxygen generation.

TGF-β also critically modulates fibroblast phenotype and function [30]. TGF-β stimulation induces myofibroblast differentiation [31] and enhances extracellular matrix protein synthesis. In addition, TGF-β exerts potent matrix-preserving actions by suppressing the activity of Matrix Metalloproteinases (MMP) and by inducing synthesis of protease inhibitors, such as PAI-1 and TIMPs [30], [1]. In addition, TGF-β is a potent inducer of Connective Tissue Growth Factor (CTGF), a fibrogenic mediator that acts in concert with TGF-β to promote persistent fibrosis [32].

The effects of TGF-β on endothelial cells are complex and dependent on the presence of contextual and environmental cues [33], [34], [35]. TGF-β is involved in the development of the vascular system by modulating the function of both endothelial cells and pericytes [33]. TGF-β has been described as being either angiogenic or angiostatic *in vivo*, depending on the nature of the assay used [33]. Beyond its effects on inflammatory and mesenchymal cells, TGF-β also modulates cardiomyocyte phenotype. TGF-β1 stimulation induces hypertrophic effects on cardiomyocytes promoting synthesis of fetal contractile proteins [36].

Expression and regulation of TGF-β in animal models of heart failure

Myocardial TGF-β synthesis is markedly and consistently upregulated in animal models of heart failure; its induction is associated with cardiac hypertrophy and fibrosis. In experimental models of myocardial infarction TGF-β isoforms demonstrate distinct patterns of regulation: TGF-β1 and β2 are upregulated early, whereas TGF-β3 exhibits a delayed and prolonged induction [37], [38]. In the pressure-overloaded myocardium TGF-β1 levels increase significantly during hypertrophic growth [39]. Angiotensin II signaling appears to play an important role in mediating TGF-β upregulation in the infarcted and remodeling myocardium [40], [41], [42]. Although extensive evidence has documented TGF-β mRNA and protein induction in the remodeling myocardium, direct demonstration of increased TGF-β activity is lacking. However, observations showing increased synthesis of TGF-β activators, such as TSP-1 [43], and enhanced phosphorylation of Smad2/3 in the infarcted

and pressure-overloaded myocardium suggest that increased TGF-β levels in the remodeling heart are associated with activation of TGF-β signaling [44], [45],

The role of TGF-β signaling in the failing heart

Evolving evidence suggests that, through its pleiotropic and multifunctional actions, TGF-β is critically involved in cardiac injury, repair and remodeling. Although TGF-β1, -β2 and -β3 exhibit distinct patterns of regulation in infarcted and hypertrophic hearts, the specific role of these isoforms in modulating myocardial remodeling remains unknown.

The in vivo effects of TGF-β1 in promoting myocardial hypertrophy and fibrosis are supported by overexpression experiments in transgenic mice. TGF-β1–overexpressing mice exhibited significant cardiac hypertrophy accompanied by interstitial fibrosis [46]. On the other hand, cardiac-restricted expression of a mutant TGF-β1 that inhibits covalent tethering of the TGF-β latent complex to the extracellular matrix thereby enhancing local TGF-β activity, was associated with atrial but not ventricular fibrosis [47], suggesting increased susceptibility of the atrial myocardium to the fibrogenic actions of TGF-β. Fibrotic remodeling of the atrium in this transgenic model was sufficient to increase vulnerability to atrial fibrillation [48].

Several investigations have demonstrated that the fibrogenic and hypertrophic actions of endogenous TGF-β may be involved in the pathogenesis of cardiomyopathic conditions. Heterozygous TGF-β1 +/− mice were protected from the development of aging-associated cardiac fibrosis and diastolic dysfunction [49]. Moreover, TGF-β receptor antagonism attenuated myocardial fibrosis in mice with an inflammatory cardiomyopathy due to cardiac-restricted Tumor Necrosis Factor (TNF)-α overexpression [50]. In the pressure overloaded heart TGF-β signaling appears to regulate matrix metabolism and cardiac hypertrophy. Administration of an anti-TGF-β neutralizing antibody prevented collagen accumulation following pressure overload and attenuated diastolic dysfunction without affecting cardiac hypertrophy [51]. However, other studies have suggested that TGF-β blockade may have deleterious effects on the pressure-overloaded myocardium. Inhibition of TGF-β signaling using mice overexpressing an inducible dominant-negative mutation of the TGF-β type II receptor was associated with markedly reduced collagen deposition following pressure overload, resulting in increased left ventricular dilation and systolic dysfunction [52]. Thus, the functional effects of TGF-β signaling on the pressure-overloaded heart are likely dependent on the level of active TGF-β. Excessive TGF-β may be deleterious by promoting collagen deposition, increased myocardial stiffness and diastolic dysfunction. However, a baseline level of TGF-β signaling may be necessary to preserve cardiac structure and to protect the pressure-overloaded myocardium from uncontrolled matrix-degradation that could result in cardiac dilation (Figure 3).

In the infarcted heart TGF-β plays a critical role in the pathogenesis of cardiac remodeling through effects on the inflammatory and reparative response [53]. Experimental studies suggest that TGF-β may be the “master switch” that mediates the transition from inflammation to scar formation following myocardial infarction (Figure 3). TGF-β signaling may suppress inflammatory cytokine and chemokine expression by deactivating macrophages, while promoting myofibroblast transdifferentiation and matrix preservation. This concept was supported by TGF-β inhibition experiments. Early TGF-β antagonism through transfection with the extracellular domain of the TGF-β type II receptor into the skeletal muscle within 24h following coronary occlusion resulted in increased mortality, enhanced neutrophil infiltration and increased pro-inflammatory cytokine and chemokine gene expression [54]. Moreover, early treatment with a neutralizing anti-TGF-β antibody also increased mortality and accentuated MMP expression in the infarcted heart [55]. In

contrast, late TGF-β inhibition after the inflammatory phase of infarct healing attenuated adverse remodeling, resulting in decreased collagen deposition; surprisingly the number of myofibroblasts in the wound was increased [56].

Recent studies suggested that the fibrogenic, but not the anti-inflammatory actions of TGF-β are mediated through Smad3 signaling. Mice with targeted disruption of Smad3 had no defect in resolution of inflammation, but exhibited reduced fibrosis in the infarct border zone and in the remodeling myocardium resulting in attenuated diastolic dysfunction [44]. In vivo and in vitro studies demonstrated that Smad3 was critically involved in myofibroblast transdifferentiation and mediated TGF-β-induced extracellular matrix synthesis and TIMP upregulation [44], [57]. Smad3 null cardiac fibroblasts were hyperproliferative, but exhibited reduced synthetic capacity and impaired contractile function [57].

The role of non-canonical TGF-β signaling pathways in cardiac remodeling remains unknown. TAK1 is activated in the myocardium after pressure overload and an activating TAK1 mutation expressed in the myocardium of transgenic mice induces cardiac hypertrophy, fibrosis and severe systolic dysfunction [58]. However, the role of endogenous TAK1 signaling in the remodeling myocardium has not been investigated. Moreover, evidence suggests that the Smad-independent TGF-β targets p-21-activated kinase 2 (PAK2) and c-Abl are involved in the pathogenesis of renal and pulmonary fibrosis [59], [60]. Whether these pathways are involved in cardiac fibrosis and remodeling is unknown.

Beyond its direct effects on the remodeling heart, TGF-β may also act by inducing expression of downstream effectors, such as CTGF and endothelin [61]. TGF-β-induced CTGF may promote cardiomyocyte hypertrophy in the remodeling heart [62] and may enhance fibrosis through synergistic interactions with TGF-β [57]. To what extent the actions of TGF-β on the remodeling myocardium are mediated through CTGF signaling remains unknown.

The relation between TGF-β and the renin-angiotensin system (RAS)

Clinical trials have documented the beneficial effects of angiotensin converting enzyme (ACE) inhibition and angiotensin II type I (AT1) receptor blockade in patients with myocardial infarction and heart failure [63], while experimental studies have established the importance of the RAS in cardiac remodeling. Angiotensin II promotes cardiomyocyte hypertrophy [64] and stimulates fibroblast proliferation and expression of extracellular matrix proteins [65], through AT1-dependent interactions. Extensive evidence suggests a direct link between the RAS system and TGF-β, indicating that TGF-β1 acts downstream of Angiotensin II [65]. Angiotensin II stimulation induces TGF-β1 mRNA and protein expression by cardiomyocytes and cardiac fibroblasts [66], [67]. Treatment with ACE inhibitors or AT1 receptor blockers markedly decreased TGF-β1 levels in hypertrophied [68] and infarcted hearts [41], [40] suggesting that TGF-β induction in the remodeling myocardium is, at least in part, mediated through angiotensin II. Schultz and co-workers demonstrated that TGF-β1 −/− mice bred in an immunocompromised Rag1 −/− background (in order to overcome the lethal consequences of TGF-β1 loss in mice, resulting in diffuse multiorgan inflammation) were protected from the development of cardiac hypertrophy in response to subpressor doses of Angiotensin II [69]. These experiments provided the first direct evidence suggesting that TGF-β1 acts downstream of Angiotensin II to promote cardiomyocyte growth.

Increased expression of TGF-β in human cardiomyopathy

Numerous studies have demonstrated that both systemic and myocardial TGF-β levels are increased in patients with cardiomyopathic conditions. Patients with dilated cardiomyopathy

exhibited increased myocardial TGF-β1 and TGF-β2 expression [70]. Higher myocardial TGF-β1 levels were associated with absence of recovery in patients with dilated cardiomyopathy undergoing left ventricular assist device implantation [71]. Cardiac TGF-β was also upregulated in patients with myocardial hypertrophy due to idiopathic hypertrophic cardiomyopathy [72], or aortic stenosis [73]; TGF-β levels correlated with the development of fibrosis [74]. An early increase in TGF-β expression was observed in atrial tissue from patients with atrial fibrillation [75]. Although these studies document induction of TGF-β isoforms in human cardiac hypertrophy and fibrosis, the significance of the findings is unclear due to the absence of data demonstrating bioactive TGF-β in the remodeling heart. Recent investigations have suggested that plasma TGF-β levels may be useful indicators of the severity of disease in patients with cardiac hypertrophy. Serum TGF-β1 levels correlated with left ventricular mass in hypertensive patients [76], whereas in patients with aortic stenosis increased levels of TGF-β1 were associated with higher transvalvular gradients and worse hypertrophy [77].

Targeting the TGF-β system in the failing heart

Because of its critical involvement in cardiac remodeling, the TGF-β system is a promising therapeutic target for myocardial infarction and for cardiomyopathic conditions associated with fibrosis and hypertrophy [61]. Late, but not early, TGF-β blockade or ALK5 inhibition [78] attenuated remodeling in experimental models of myocardial infarction. In addition, TGF-β inhibition through administration of inhibitory peptides [79], or neutralizing antibodies [51], prevented the development of cardiac fibrosis in animals with pressure overload. Moreover, human cardiomyopathic conditions are associated with intense activation of the TGF-β system; TGF-beta levels often reflect the development of cardiac remodeling [77].

Although these findings support the use of TGF-β/ALK5 inhibitors to protect from the development of cardiac remodeling, translation of these strategies in the human pathologic conditions is challenging due to the broad range of effects of TGF-β and its important role in tissue homeostasis. Recent studies have suggested that TGF-β1 exerts antiatherogenic actions [80]; thus, chronic inhibition of TGF-β signaling may accelerate plaque formation. In addition, excessive TGF-β inhibition may result in abrogation of essential matrix-preserving pathways, leading to unopposed matrix degradation, cardiac dilation and dysfunction.

Prolonged inhibition of the TGF-β system in patients with chronic cardiomyopathy in order to attenuate hypertrophy and fibrosis will also likely interfere with immune regulation. Thus, identification of a suitable therapeutic target for patients with cardiomyopathy requires dissection of the pathways responsible for the fibrogenic and immunomodulatory effects of TGF-β in order to selectively inhibit profibrotic signaling. In the infarcted myocardium, on the other hand, the effects of short term inhibition of TGF-β signaling may be dependent on the timing of the intervention. TGF-β inhibition during the inflammatory phase of infarct healing appears to be detrimental and may result in exacerbated and prolonged local inflammatory response and in accentuated matrix degradation. Late TGF-β inhibition, on the other hand, does not interfere with resolution of the inflammatory reaction and may exert beneficial actions through attenuation of fibrotic and hypertrophic remodeling.

Because of the wide range of actions mediated by TGF-β, dissection of the signaling pathways responsible for specific effects would greatly benefit our efforts to identify optimal therapeutic targets. Our recent work has suggested that Smad3 critically regulates the fibrogenic actions of TGF-β, without affecting the time course of resolution of the inflammatory response [44], [57]. Because Smad3 signaling is selectively activated in the

infarct border zone, Smad3 inhibition may protect from fibrotic remodeling of the ventricle while avoiding other unwanted effects of TGF-β blockade. Thus, our current understanding of the role of TGF-β signaling in the remodeling heart suggests that short-term inhibition of the pro-fibrotic Smad3 pathway during the reparative phase of infarct healing may be a reasonable strategy to prevent expansion of fibrosis. However, a word of caution should be raised regarding the applicability of these insights, derived from experimental studies using young animals, to elderly patients. Our recent work has demonstrated that, in contrast to young animals, senescent mice exhibit markedly reduced collagen deposition in the scar associated with increased chamber dilation. Moreover, we found that fibroblasts isolated from aged hearts have impaired responses to TGF-β [81], [82]. Thus, whether the beneficial effects of Smad3 inhibition on the infarcted heart extend to senescent subjects remains unknown.

Future directions

Despite extensive evidence suggesting an essential role for TGF-β in heart failure and cardiac remodeling, translation of the findings in human patients is hampered by challenges due to the unique biology of TGF-β activation and the pleiotropic actions of TGF-β signaling. Global inhibition of TGF-β signaling would be expected to have unwanted effects both on the cardiovascular system and in other organs. Thus, in order to therapeutically exploit the TGF-β system in heart failure new knowledge in several important areas is needed:

1. Dissection of the pathways responsible for specific TGF-β-mediated effects on the failing heart. Understanding the signals involved in transducing the fibrogenic and hypertrophic actions of TGF-β will allow us to design selective therapies to prevent adverse cardiac remodeling.
2. Understanding of the spatial and temporal characteristics of TGF-β signaling. Because of the potential role of TGF-β in resolution of inflammation, optimal timing of therapeutic interventions targeting the TGF-β system in patients with myocardial infarction is critical. In addition, because of the distinct roles of matrix deposition in formation of a supportive scar and in fibrotic remodeling of the non-infarcted myocardium, the consequences of TGF-β inhibition during the fibrotic phase of infarct healing may depend on the topographic localization of the inhibitory agent.
3. Study of the effects of TGF-β on various cell types involved in cardiac remodeling. Understanding the *in vivo* significance of TGF-β-mediated actions on specific cell types may allow us to predict the consequences of TGF-β inhibition on the remodeling heart. Generation of mutant mice with selective disruption of the TGF-β response in specific cell types may provide important information on the mechanisms of TGF-β mediated effects.
4. Understanding of the mechanisms responsible for TGF-β activation in the remodeling heart.
5. Dissection of the distinct roles of TGF-β1, β2 and β3 in cardiac remodeling.
6. Understanding of the impact of current pharmacologic therapies for heart failure on the TGF-β system. Because of the link between the RAS, β-adrenergic blockade and TGF-β signaling, current pharmacologic treatment may act, at least in part, through attenuation of TGF-β. Thus, additional benefit from TGF-β inhibition may be relatively modest.

7. Study of the role of TGF-β signaling in high-risk populations, such as the diabetics and the elderly. Most of our understanding of the role of TGF-β in cardiac remodeling is derived from studies performed in young animals. Extrapolation of the findings in elderly patients with heart failure, or in individuals with concomitant illnesses (such as diabetes), may be problematic. The impairment in TGF-β/Smad3 signaling observed in cardiac fibroblasts from senescent mice [81] highlights the importance of understanding the consequences of aging on the TGF-β system [83].

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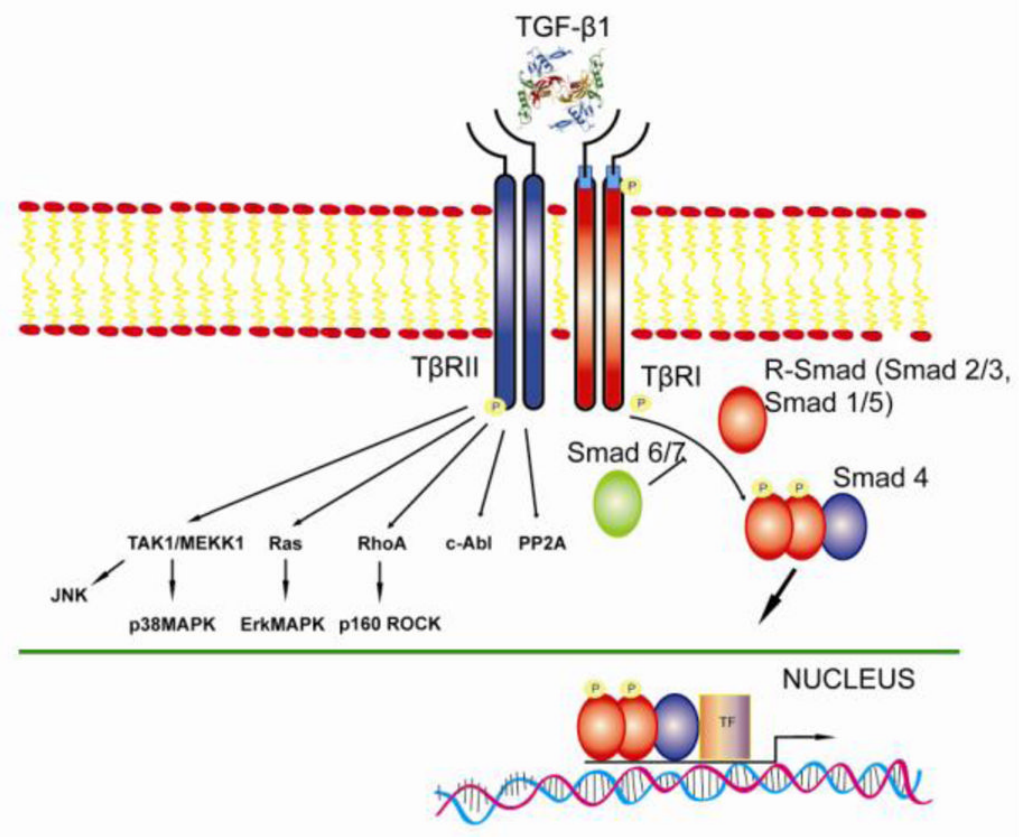

Figure 1.
TGF-β signaling pathways. TGF-β transduces its signal through Smad-dependent and Smad-independent pathways.

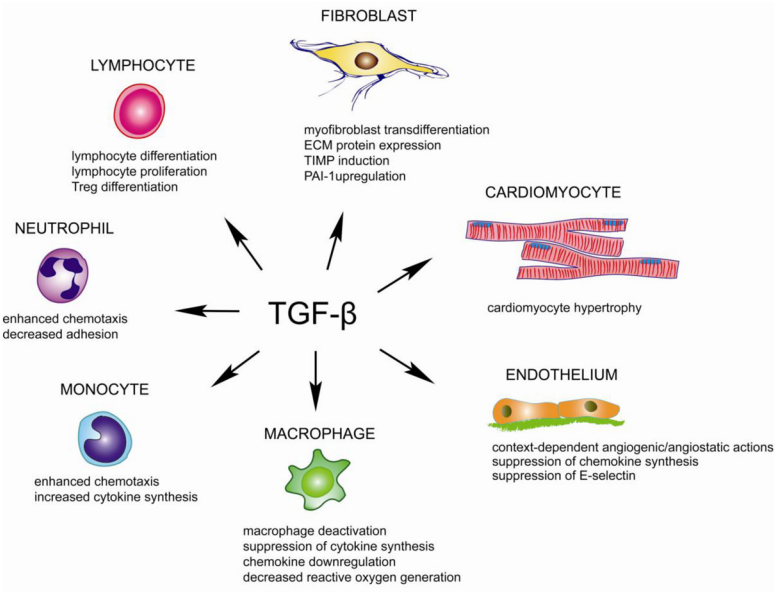

Figure 2.
The cellular effects of TGF-β. TGF-β exerts pleiotropic effects on all cell types involved in cardiac injury, repair and remodeling.

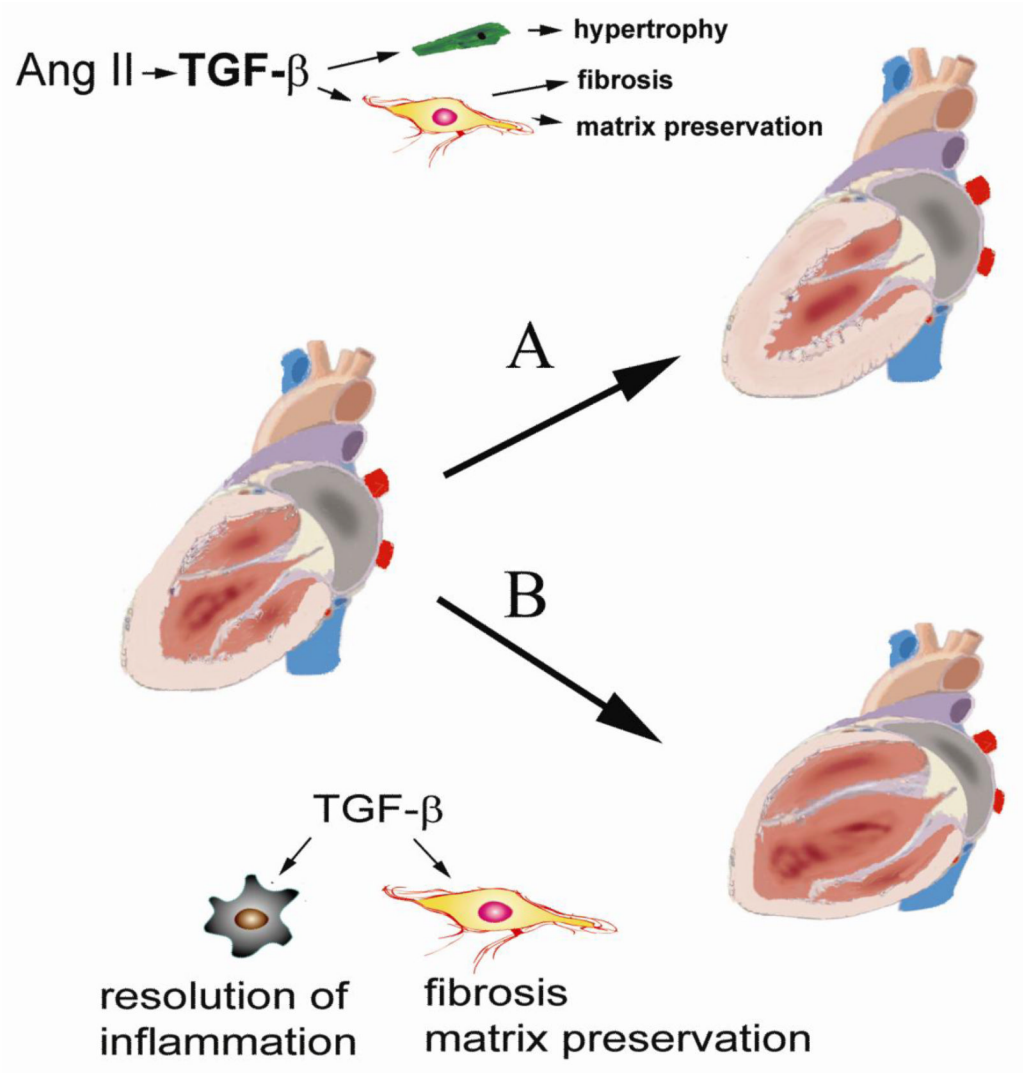

Figure 3.

The role of TGF-β in hypertrophy, fibrosis and post-infarction cardiac remodeling. A: TGF-β transduces hypertrophic signals in cardiomyocytes and induces myofibroblast transdifferentiation, while promoting matrix deposition and preservation. Although TGF-β inhibition in the pressure-overloaded ventricle may attenuate hypertrophy and reduce fibrosis protecting from diastolic dysfunction, complete loss of TGF-β signaling may result in unopposed matrix degradation, cardiac dilation and systolic dysfunction. B: In the infarcted heart TGF-β may serve as the “master switch” that regulates transition from the inflammatory phase to scar formation. TGF-β suppresses inflammatory mediator synthesis by macrophages while enhancing myofibroblast transdifferentiation and matrix deposition. Thus, timing is a crucial determinant of outcome in pharmacologic interventions targeting the TGF-β system following myocardial infarction.