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Review

Role of transforming growth factor-β in the progression of heart failure

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Abstract. Transforming growth factor (TGF-β) is a multifunctional peptide growth factor that has an important role in the regulation of cell growth, differentiation, and repair in a variety of tissues. In mammals, the cytokine has three isoforms, TGF-β1, TGF-β2, and TGF-β3. TGF-β1 is up-regulated by Ang II and induction of TGF-β1 causes cardiac fibrosis. The stimulus that triggers the expression of TGF-β1 may be repeated causing continual injury, which is associated with an increase in the activity of Ang II in heart tissue. The interplay between Ang II and TGF-β1 causes continued activation that may result in chronic hypertension and progressive myocardial fibrosis, leading to heart failure. The regulation of TGF-β1 secretion and action involves complex transcriptional events. Overproduction of TGF-β1 underlies tissue fibrosis. Understanding the actions and signaling transduction of TGF-β could lead to the development of therapeutic options that may be effective in inhibiting myocardial fibrosis triggered by TGF-β1 in heart failure.

Keywords. Transforming growth factor-β, signaling pathways, heart tissues, therapeutic options.

Introduction

Heart failure is a leading cause of disability and death, and an important contributor to the cost of health care [1–4]. Advances in cytokine biology have opened an avenue to the understanding of the molecular events underlying chronic heart failure. It has become clear that cardiac remodeling is attended by cardiac hypertrophy and interstitial fibrosis, leading to the loss of normal cardiac function and heart failure [5–7]. This dynamic structural remodeling is a milestone in the progression to heart failure [8–10]. A pivotal mediator in cardiac remodeling is the activation of cytokines in response to myocardial overload and injury. Several lines of evidence point to transforming growth factor-beta (TGF-β) as a powerful cytokine that initiates and terminates tissue repair and sustained production underlies the development of myocardial fibrosis [11]. Moreover, increased TGF-β1 expression has been identified in the myocardium during cardiac hypertrophy and heart failure [12–14]. Thus, TGF-β1 expression may directly participate in the progressive remodeling process in heart failure. This review discusses the biological actions of TGF-β, focusing its role in the progression of heart failure. Understanding the actions of TGF-β1 and its signaling transduction could lead to the potential development of antifibrotic drug as a therapy in addition to conventional treatments in heart failure.

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TGF-β isoforms: structure, function and synthesis

Based on structural and biological similarities, the TGF-β superfamily can be subdivided into four major families: the Mullerian inhibitory substance (MIS) family, the

inhibin/activin family, the bone morphogenetic protein (BMP) family, and the TGF-β family [15]. MIS can induce regression of the Mullerian duct in male embryos [16]. The inhibins and activins were originally identified by their ability to regulate hormone secretion in pituitary cells [17]. BMPs were purified as factors that induce ectopic bone formation and they regulate various early developmental processes in invertebrates and vertebrates [18].

Five distinct members of TGF-β family have been identified in vertebrates; three structurally and functionally similar TGF-β isoforms are expressed in mammals (TGF-β1, 2, and 3) [15, 19]. The TGF-β1, TGF-β2, and TGF-β3 genes have been mapped by distinct genes on chromosomes 19q13.1-q13.3 19, 1q41 [20], and 14q23-24 [21], respectively. TGF-β is a prototypical, multifunctional peptide growth that was isolated from platelets and its characteristic was known just over two decades ago [11]. Distinguished initially for their ability to inhibit the growth of most epithelial and hematopoietic cells, and to regulate the production of extracellular matrix (ECM) by mesenchymal cells, these peptides are now known to control a great diversity of fundamental biological processes such as cell growth, differentiation, development, tissue repair, and apoptosis [22–24]. Virtually every cell in the body, including epithelial, endothelial, hematopoietic, neuronal, and connective tissue cells, produces TGF-β and has receptors for it. TGF-β1 mRNA is expressed in endothelial, hematopoietic, and connective tissue cells. Thus, it is the isoform most implicated in tissue fibrosis, including the fibrotic disease of the heart, kidney, liver and lung [11]. TGF-β2 mRNA is mostly expressed in epithelial and neuronal cell, and TGF-β3 mRNA primarily in mesenchymal cells [25].

Loss of function studies with TGF-β family ligands in mice has also demonstrated their multifunctional properties and revealed their important role during embryogenesis and in maintaining homeostasis during adult life [26]. TGF-β1 knockout animals have over 50% embryonic lethality associated with defects in early hematopoiesis and vasculature in the yolk sac [27]. Shull et al. [28] have shown that animals homozygous for the mutated TGF-β1 allele have no gross developmental abnormalities, but about 20 days after birth they succumb to a wasting syndrome accompanied by a multifocal, mixed inflammatory cell response and tissue necrosis, leading to organ failure and death. Moreover, Kulkarni et al. [29] have demonstrated that TGF-β1 null mice reveal an excessive inflammatory response with massive infiltration of lymphocytes and macrophages in many organs, but primarily in heart and lungs [29]. Mice deficient in the TGF-β2 gene die around birth and show developmental defects in areas including the heart, lung, limbs, spinal column, eye, inner ear, and urogenital system [30, 31]. The TGF-β3 knockout phenotype is characterized by cleft palate and delayed pulmonary development [32]. The distinct phenotypes of TGF-β1, 2, and 3 knockout mice provide evidence that these TGF-β isoforms play distinctive roles in embryonic growth and development [24].

In fibrotic diseases of all three isoforms, regions of increased matrix show increased of TGF-β, especially the isoform of TGF-β1 [11, 33]. The TGF-β1 encodes a 390-amino acid precursor molecule that contains a signal peptide, the active TGF-β1 molecule, and a latency-associated peptide (LAP) [34]. After removal of the signal peptide, the TGF-β1 gene product is proteolytically cleaved to form mature TGF-β1 and the latency associated peptide [35]. Before secretion, TGF-β1 non-covalently associates with LAP to produce an inactive latent TGF-β1 complex [36, 37]. TGF-β1 can be released from the latent complex, and thereby activated by changes in pH, by proteases such as plasmin and cathepsin D, and by thrombospondin [38, 39]. Once activated, TGF-β1 is capable of binding a cell surface receptor, thereby initiating an intracellular signaling cascade (Fig. 1).

Molecular mechanism of TGF-β1 signal transduction

The pathway of TGF-β signaling is summarized in Figure 1. The three isoform of TGF-βs are synthesized as precursor protein from TGF-β messenger RNA, which are biologically inactive or latent form [15, 40, 41] containing an LAP. Latent TGF-β undergoes activation with release of the LAP. TGF-β in turn binds to three high-affinity cell surface receptors known as types I, II, and III. Type III receptors are the most abundant type. They bind to TGF-β and then transfer it to its signaling receptors, the type I and II receptors. The expression of the TGF-β receptors represents another mechanism for regulating the activity of TGF-β activity [15]. Endoglin, another TGF-β receptor that is abundant on endothelial cells, contains a transmembrane region and a cytoplasmic tail homologous to the type III receptor.

Intracellular signaling of TGF-β occurs via two receptor serine/threonine kinases, type I and type II receptors. The active form of TGF-β binds to type II receptor, which is followed by the recruitment of the type I receptors to form tetrameric complexes with the type II receptors [42]. After receptor activation, the signal is propagated downstream through the recently identified Smad protein family [42, 43]. In the mammalian heart, Smads can be divided into three major groups: the receptor-regulated Smads (R-Smads: Smad 1, Smad 2, Smad 3, Smad 5, and Smad 8), common-mediator Smad (Co-Smad: Smad 4), and inhibitory Smads (I-Smad: Smad 6 and Smad 7) [42]. Smads 1, 5, and 8 serve principally as substrates for the BMP and anti-Mullerian receptors, and Smads 2 and 3 for the TGF-β, activin, and nodal receptors [44].

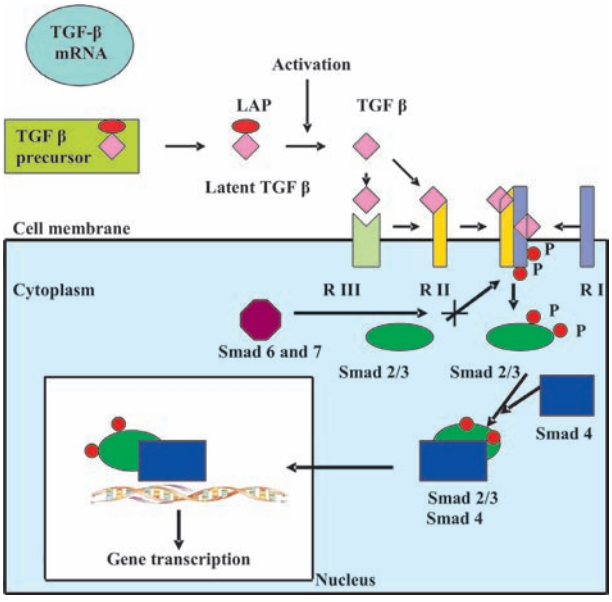

Figure 1. The activation and signal transduction of TGF-*β*. Each isoform of TGF-*β* from TGF-*β* messenger RNA is initially synthesized as a precursor molecule. The latency-associated peptide (LAP) is cleaved from TGF-*β* by proteolytic processing. The dissociation/release of this peptide renders TGF-*β* biologically active. TGF-*β* binds to the type III receptor, transferring it to its signaling receptors, type II receptors. This leads to recruitment of the type I receptor. Activation of type II and type I receptors result in phosphorylation of Smad 2 and Smad 3. Smad 2 and Smad 3 then form a complex with Smad 4. The complex translocates to the nucleus, and activates target genes. Smad 6 and 7 block the phosphorylation of Smad 2 and Smad 3 (RIII, type III receptor; RII, type II receptor; RI, type I receptor).

Activation of the type I receptor results in phosphorylation of the pathway-restricted R Smads, which then form a heteromeric complex with Co-Smad [45]. Co-Smad is a common partner for all of the receptor-activated R-Smads [46]. The R-Smad-Co-Smad complex translocates to the nucleus where, either alone or in association with a DNA-binding subunit, it activates target genes by binding to specific promoter elements. TGF-*β* family members induce concentration-dependent responses that may be due to activation of promoters with different affinities for Smad-containing transcription factor complexes along a concentration gradient of TGF-*β* [47]. Smad 6 and Smad 7 function as inhibitors of TGF-*β* signaling by binding to type 1 receptors and interfering with the phosphorylation of R-Smads [46, 47], or recruit the ubiquitin ligases Smurf1 (Smad ubiquitination-related factor 1) and Smurf2 (Smad ubiquitination-related factor 2) to induce proteasomal degradation of the receptor complexes [48, 49]. They may have a negative feedback role in signal transduction. However, it is in the nucleus that the Smad complex binds to target genes as the endpoint of the TGF-*β* signal, and thus the net-R Smad signal to the nucleus is an important determinant of the TGF-*β* function [50, 51].

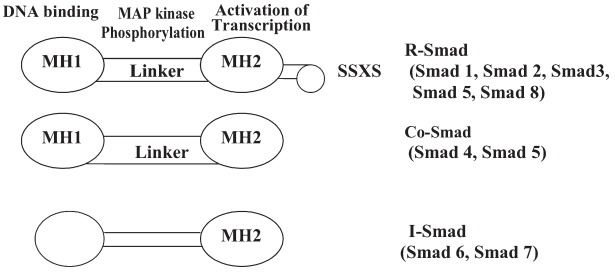

Figure 2. The structure of the Smad family. The Smad family contain conserved N-terminal and C-terminal domains known as the MH1 and MH2 domains, respectively. The globular MH1 and MH2 are coupled by a linker region (MH1, Mad-homology1; MH1, Mad-homology 2).

The structure of Smad family consists of two globular domains coupled by a linker region (Fig. 2) [24, 44]. The N-terminal domain or 'Mad homology 1' (MH1) is highly conserved in all R Smads and Co-Smad, but not in I-Smads. The MH1 domain has DNA-binding activity. The linker region contains a transcriptional activation motif and phosphorylation sites for Smurf, phosphorylation sites for several classes of protein kinases, and, in Smad 4, a nuclear export signal (NES) [44, 52]. C-terminal, or MH2, domain is conserved in all Smad proteins, which drives translocation into the nucleus and regulates transcription of target genes [24, 53]. TGF-*β*1 receptor-mediated phosphorylation of the C-terminal sequence SSXS appears to relieve these two domains from a mutually inhibitory interaction, leading to R-Smad activation. Co-Smads lack the SSXS sequence and are therefore not phosphorylated by the type 1 receptor. Their interaction with R-Smad is primarily mediated by the MH2 domain [24, 54].

Once within the nucleus, the R-Smad-Co-Smad complex may form specific interactions with nuclear transcription factors such as activating protein-1 (AP-1) [24, 52]. Both AP-1 and Smads mediate enhanced expression of TGF-*β* responsive genes, such as collagen [55], c-Jun [56], or endothelin-1 [57]. These genes have important functions in ventricular remodeling or in vascular angiogenesis. AP-1/Smads signaling is also involved in the induction of apoptosis in TGF-*β*-stimulated cells. Furthermore, the simultaneous activation of AP-1 and Smads after myocardial infarction (MI) may be the trigger for fibrosis and apoptosis in the heart [24], suggesting that AP-1/Smads signaling plays a central role in deteriorating remodeling process in post-infarction heart failure.

Alternative signaling pathways

Recent studies have shown that there is extensive cross-talk between the TGF-*β*-Smad pathway and other signaling pathways [24] (Fig. 3). In addition to this Smad

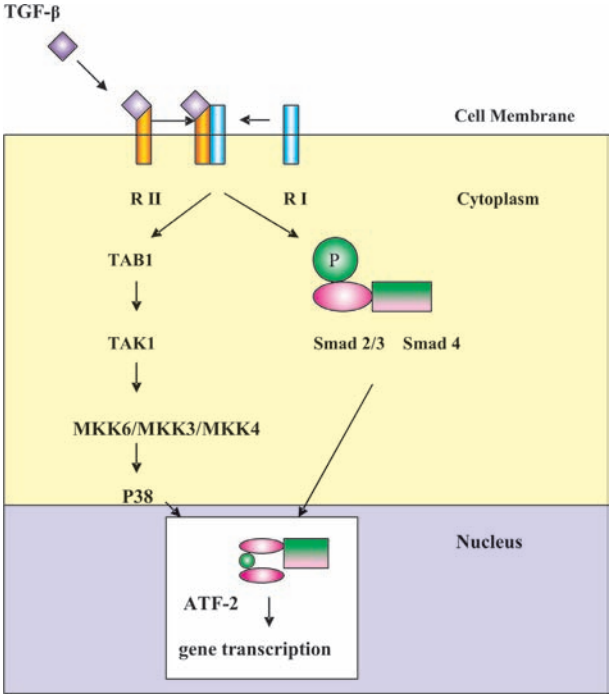

Figure 3. Schematic representation of cross-talk between TGF-β-Smad signaling pathway and the MAPK signaling cascades. Upon binding of TGF-β to the type II receptor, the TGF-β-bound type II receptor forms a heteromeric complex with the type I receptor, resulting in the activation of the latter's serine/threonine kinase activity. The activated type I receptor then phosphorylates the bound Smad 3 or Smad 2 protein, which results in its release from the type I receptor. The released Smad 2 or 3 forms a hetero-oligomer and moves to the nucleus. This hetero-oligomer directly binds to ATF-2, resulting in enhanced ATF-2 activity. In addition to this Smad pathway, the TAK1 pathway is required for TGF-β signal transduction. Upon TGF-β stimulation, the TAB1 protein is activated, resulting in its binding to the serine/threonine kinase domain of TAK1. The TAK1 activates MKK3, MKK6 and MKK4 of the MAPKK family. TAK1 activates p38, one member of MAPK family via MKK6/MKK3 and possibly through MKK4. p38 directly phosphorylates ATF-2, resulting in enhancement of the trans-activity capacity of ATF-2 (ATF, activating transcription factor; TAK, TGF-β activated kinase; TAB, TAK1-binding protein; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; RII, type II receptor; RI, type 1 receptor).

pathway, the TGF-β1-activated kinase (TAK1) pathway is required for TGF-β signal transduction. Upon TGF-β stimulation, the TAB1 protein is activated, resulting in its binding to the serine/threonine kinase domain of TAK1 [58]. TAK1 then activates MKK3, MKK6 and MKK4 of the MAPKK family. TAK1 activates p38, one member of mitogen-activated protein kinase (MAPK) family via MKK6/MKK3 [59] and possibly through MKK4. p38 directly phosphorylates ATF-2, resulting in enhancement of the trans-activity capacity of ATF-2 [60]. Thus, the Smad and TAK1 pathways synergistically stimulate TGF-β-induced transcription.

In the heart, MAPKs such as extracellular receptor-linked kinase (ERK), c-Jun N terminal kinase (JNK), and p38 are expressed [61–63]. In heart failure of ischemic or nonischemic origin, both the sympathetic nervous system and the renin-angiotensin system are activated [60, 64, 65]. Recently, Schulz et al. [61] reported that p38 and JNK MAPK phosphorylation are increased, as are p38 MAPK-α and-β activities, but the ERK MAPK phosphorylation remains unaltered in the model of pacing-induced heart failure in rabbits. Using the same animal model, Qin et al. [66] showed that MAPK activation occurred at the early stage of heart failure. Furthermore, Haq et al. [67] reported that all three MAPKs (ERK, JNK and p38) were activated in patients with advanced heart failure, irrespective of ischemic or idiopathic cardiomyopathy. More recently, Matsumoto-Ida et al. [68] have shown that TGF-β–TAK1–MKK3/6-p38 MAPK pathways in the cardiomyocytes of non-infarcted spared myocardium are activated after acute MI, and may play a role in ventricular hypertrophy and post-MI remodeling in rats. Using a post-MI rat model, See et al. [69] demonstrated that p38 MAPK inhibitor had beneficial effects on left ventricular (LV) remodeling and dysfunction after MI. These findings demonstrate that p38 MAPK plays a role in mediating TGF-β1 induced myofibroblast activation, and may contribute directly to cardiac remodeling and fibrosis in post-MI.

TGF-β1 as pathological mediator of heart failure

TGF-β1 has numerous actions on the ECM [13]. It acts on cells to induce the deposition of ECM by simultaneously stimulating cells to increase several fold the synthesis of most matrix proteins, decreasing the production of matrix degrading proteolytic enzyme, increasing the production of inhibitors of these proteases, and modulating the expression of integrins in a manner that increases cellular adhesions to the matrix [11, 17]. As a result, this powerful fibrogenic cytokine is increasingly recognized as an important growth factor in mediating myocardial fibrosis (Fig. 4).

In the heart, TGF-β is induced by injury [70], pressure overload [71], angiotensin II (Ang II) infusion [72], norepinephrine infusion [73], and inhibition of nitric oxide (NO) [74], during which concomitant expression of collagen and fibronectin is observed [71, 72]. TGF-β1 is present in both cardiomyocytes and myocardial fibroblasts [75, 76], and has been implicated in cardiomyocyte growth [77], fibrosis [74, 78–82] and re-expression of the fetal isoforms of myofibrillar protein genes [82, 83], all characteristic of cardiac remodeling. In a recent study, Shultz et al. [84] have shown that TGF-β1 has a direct action on cardiac remodeling response of the heart to Ang II.

Cardiac hypertrophy induced by pressure overload is accompanied by increases in the deposition of ECM

proteins [84]. Villarrel et al. [77] showed that elevated TGF-β1 levels increased contractile protein synthesis in cultured adult rat cardiomyocytes and ECM production in cultured myocardial fibroblasts. Animal studies suggest that hypertrophic stimuli (pressure overload or norepinephrine) increase TGF-β1 gene expression in hypertrophic myocardium [85, 86]. Using the transgenic mice, Rosenkranz et al. [87] demonstrated that TGF-β1 induced cardiac hypertrophy and enhanced β-adrenergic signaling *in vivo*. Thus, these data support the view that TGF-beta 1, released by myocytes and acting in an autocrine and/or paracrine manner, is involved in myocardial remodeling by hypertrophic stimuli.

TGF-β1 may be important in dilated cardiomyopathy in which there is an overall increase in intramyocardial fibrillar collagen. Pauschinger et al. [88] presented results of 30 patients with dilated cardiomyopathy suggesting that the gene expression of collagen type I and collagen type III correlated with the gene expression of TGF-β1 in myocardial biopsy specimens. Sanderson et al. [89] demonstrated that in patients with idiopathic dilated cardiomyopathy increased macrophage gene expression for TGF-β1 associated with increased circulating concentrations of TGF-β1. Moreover, Li et al. [90] have shown that gene expression of TGF-β1 is enhanced in patients with idiopathic hypertrophic cardiomyopathy, and may be associated with its development.

MI is a common etiology for the development of heart failure [91, 92]. After MI, the myocardium undergoes a repair process involving scar formation at the site of injury that includes fibroblast and myofibroblast and concomitant deposition of ECM proteins [14, 93]. Cardiac fibrosis also occurs in non-infarcted segments of myocardium. Further expansion of the ECM impairs diastolic stiffness and compromises systolic mechanics, contributing to subsequent cardiac hypertrophy and heart failure [5, 6] (Fig. 3). In this regard, the expression of TGF-β1 and up-regulated TGF-β1 receptor expression [94] in the myocardium are the powerful synthesis of collagen.

Stimuli for TGF-β1 expression in heart failure

Ang II and TGF-β1 play an important role in progressive cardiac fibrosis during cardiac hypertrophy and heart failure [13, 14]. In this respect, Ang II stimulates cardiomyocyte hypertrophy by paracrine release of TGF-β1 via AT_1 receptor binding in neonatal cell culture model [95, 96] and *in vivo* (Ang II infusion) study [97]. Ang II increased TGF-β1 expression in cardiac fibroblasts, which may act as an autocrine/paracrine stimulus for collagen formation. Type I and III collagens are the major fibrillar collagens produced by fibroblasts in the adult heart [30], and appear to account for most of the myocardial fibrosis following injury [97]. Fibrillar collagen of the heart provides the structural scaffolding for cardiomyocytes and coronary vessels and imparts cardiac tissue with physical properties that include stiffness and resistance to deformation [98].

Recently, a major advance in understanding TGF-β1 post-receptor signaling is the identification of Smad proteins as effector proteins. Hao et al. [99] found that activation of TGF-β1 and the increased expression of novel downstream Smad 2 and Smad 4 signaling proteins in infarct scar and remnant myocardium occurred during the chronic phase of MI, and angiotensin was shown to

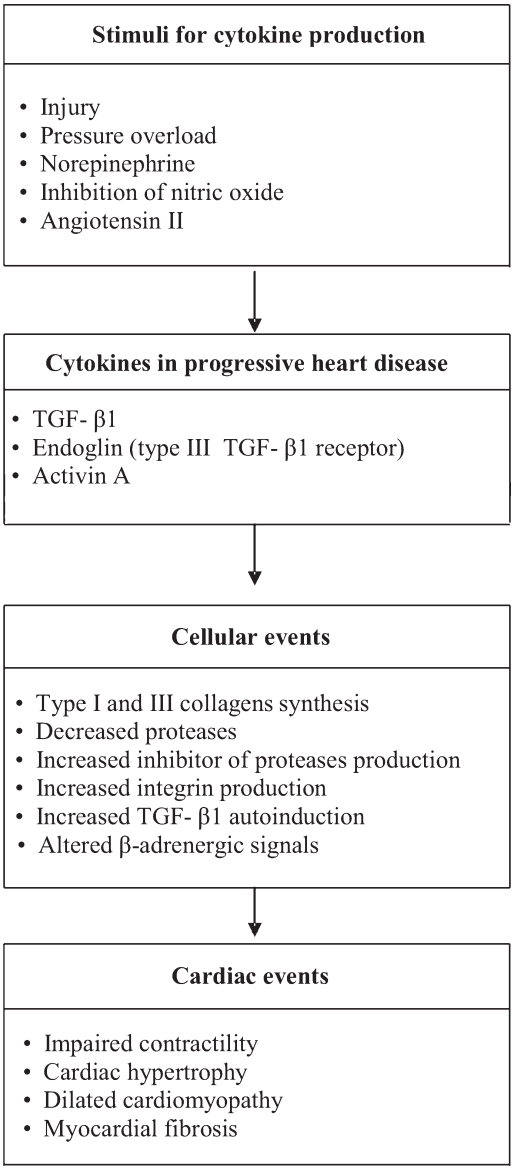

Figure 4. Potential mechanisms that are involved in the development of heart failure. The TGF-β1 gene and endoglin are up-regulated in response to myocardial overload and injury. Cytokine TGF-β1 may induce the synthesis and deposition of ECM and contribute to structural cardiac remodeling involving cardiomyocyte hypertrophy and myocardial fibrosis. This in turn contributes to negatively contractility that ultimately leads to heart failure.

elevate Smad 2 expression. This study suggests the cross-talk between angiotensin and Smad signaling is associated with fibrotic events in post-MI heart failure.

TGF-β1 modulates tissue fibrosis by direct effects on fibroblast function [98]. This powerful fibrogenic cytokine has been demonstrated to mediate myocardial fibrotic response in various models of progressive cardiac remodeling [100]. Cardiac remodeling denotes alterations in the structural components of the myocardium, involving both myocyte and non-myocyte components, a process which underlies progressive heart failure [101]. In a recent study, Chen et al. [102] showed that endoglin, a type III TGF-β1 receptor, was expressed in cardiac fibroblasts, and that it was up-regulated by Ang II via AT_1 receptor. Endoglin was demonstrated as a potent mediator of profibrotic effects of Ang II on cardiac fibroblast in addition to TGF-β1 [102]. Another member of TGF-β superfamily, acitivin A, may also be up-regulated by Ang II during clinical and experimental heart failure, and induces myocardial remodeling after MI, suggesting a pathogenic role of this cytokine in the development of heart failure [103].

Given that fibrosis can be considered as resulting from a failure to suppress the normal wound healing response, clues as to how fibrosis develops might emerge from the identification of ways that mammals have of suppressing the normal wound healing process. After MI in a rat model, expression of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, TGF-β1, and TGF-β3 are increased maximally by approximately 2-fold at about 1 week after MI [102]. TNF-α can independently induce AT_1 receptor up-regulation, enhancing Ang II-mediated effects in favor of fibrosis [104]. In rodent models of MI, within the first hours to 1 day, there are robust up-regulations of intramyocardial cytokines including TNF-α, IL-1 β, and IL-6 mRNA expression in the infarct area (up to 50-fold), as well as in the non-infarct area (up to 15-fold) [105–107]. This robust up-regulation may turn to baseline level if the infarct is small. However, if the infarction is large, or if the host's inflammatory response is exuberant, there can be either sustained cytokine up-regulation or a second wave of cytokine up-regulation, mediating important chronic remodeling phase. The elevation in cytokine expression precedes the consequent increase in local matrix metalloprotein (MMP) activity (*e.g.* MMP-2 and -9) in the infarct area and collagen expression in the non-infarcted myocardium [105, 107].

TGF-β induces the surviving cells to produce ECM and additional TGF-β. Furthermore, expression of all three isoforms are detected in many different cell types during repair, with each isoform having a characteristic distribution in the wound tissue [108]. In most studies, a rapid induction of TGF-β1 and TGF-β2 was observed, whereas an increase in TGF-β3 expression was seen at the later stages of repair [107, 108]. In addition to the TGF-β ligands, the type I and type II TGF-β receptors are present in various cell types within the endothelium at the wound edge [109]. Collectively, these findings strongly suggest that TGF-β plays a critical role during the healing process after MI and repeated tissue injury. A defect in TGF-β regulation, or both lead to the continuous production of TGF-β and ECM, resulting in myocardial fibrosis through vicious cycle [11, 110] (Fig. 4).

Mechanisms of TGF-β1 and Ang II interaction

The causal link between Ang II and TGF-β1 in cardiac hypertrophy and fibrosis has been recently proven [84]. The lack of the TGF-β1 gene fully prevented the development of cardiac hypertrophy and dysfunction, induced by suppressor doses of Ang II, that were observed in wild-type mice [111]. Wenzel et al. [112] have shown that the intracellular signaling events that are required for the Ang II-dependent up-regulation of TGF-β1 mRNA in adult cardiomyocytes is mediated by NAD(P)H oxidase, and subsequent activation of protein kinase C (PKC), p 38-MAP kinase, and nuclear AP-1 (Fig. 5). Ang II-stimulated up-regulation of TGF-β1 mRNA was also shown in fibroblasts, in which it depends on transactivation of the epidermal growth factor receptor and ERK activation by Ang II. Recent studies have shown that the downstream mediators of cardiac Ang II and TGF-β1 interaction may include Smad protein, TAK1, and induction of hypertrophic responsiveness to β-adrenergic stimulation in cardiac myocytes [111].

Interactions of TGF-β1 with other growth factors

Connective tissue growth factor. Connective tissue growth factor (CTGF) is a novel and potent profibrotic factor, a cysteine-rich protein induced by TGF-β1 in connective tissue cell [113], but not in epithelial cells [114, 115]. CTGF is implicated in fibroblast proliferation, cellular adhesion, angiogenesis, and ECM synthesis [114, 116]. The regulation of CTGF appears to be controlled primarily at the level of transcription, and a brief exposure of fibroblasts to TGF-β is sufficient to induce a prolonged high level of CTGF expression. TGF-β directly induces CTGF promoter activity in fibroblasts [116, 117], strongly suggesting that CTGF gene expression may function as a downstream mediator of TGF-β action on fibroblast [118]. Both TGF-β and CTGF mRNA are overexpressed in numerous fibrotic disorders in rat model with post-infarction heart failure and in patients with cardiac ischemia, and occur in the active phase of matrix synthesis during wound repair [119]. This correlated with concomitant increases in fibronectin, and type I and type III collagen mRNA levels [120]. Thus, it

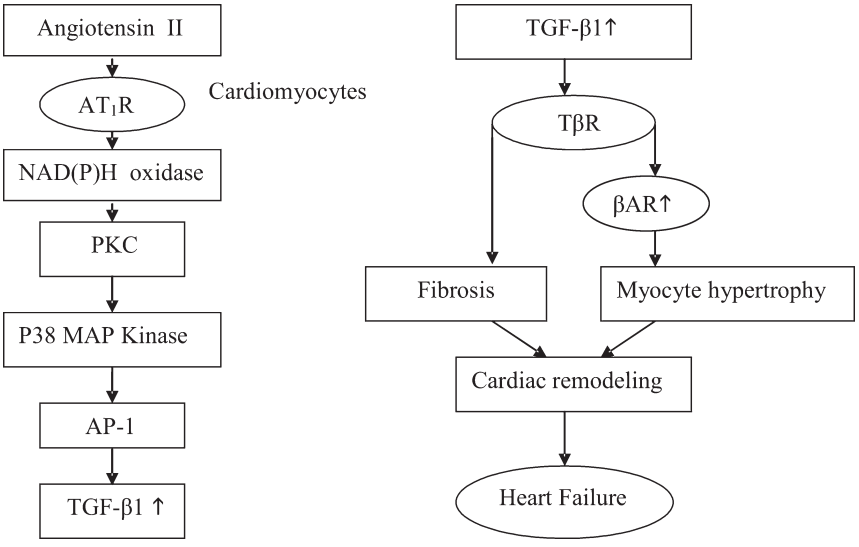

Figure 5. Intracellular signal pathways of Ang II-induced TGF-β1 expression and the mechanism of Ang II and TGF-β1 interaction. Ang II induces the expression of TGF-β1 in adult ventricular cardiomyocytes via intracellular signaling transduction. The increased Ang II-induced TGF-β1 expression in cardiomyocytes and cardiac fibroblasts stimulates myocyte hypertrophy and cardiac fibrosis. The consequence of cardiac remodeling leads to heart failure (AT1R, angiotensin type 1 receptor; NAD(P)H, nicotinamide-adenine dinucleotide phosphate; PKC, protein kinase C; AP-1, nuclear activating protein-1, TβR, TGF-β receptor; βAR, β-adrenergic receptor).

is conceivable that pharmacological inhibition of TGF-β stimulation of CTGF expression may be an effective therapeutic approach to a variety of undesirable fibrotic reactions [120]. Furthermore, using a wound model of fibrosis in rats, Duncan et al. [118] showed that blockade of CTGF action with neutralizing antibodies, or inhibition of CTGF synthesis by gene repression, significantly reduced fibroblast collagen synthesis induced by TGF-β. Whether inhibition of CTGF synthesis induced by TGF-β is essential in all fibroblasts, such as cardiac fibroblast, by CTGF-dependent pathways remains to be investigated.

Hepatocyte growth factor. Hepatocyte growth factor (HGF), originally identified and cloned as a potent mitogen for hepatocytes, regulates cell growth, cell motility, and morphogenesis of various types of cells [121, 122]. It is a unique growth factor for preventing fibrosis [123], because previous studies showed that administration of human recombinant HGF (rHGF) prevented and/or regressed fibrosis in liver and pulmonary injury models [124–127]. Furthermore, Taniyama et al. [123] demonstrated that there was a marked reduction of local HGF mRNA and concentration in the myocardium of cardiomyopathic hamsters, and blockade of Ang II significantly stimulated local HGF expression and production, accompanied by inhibition of myocardial fibrosis, because HGF stimulated the degradation pathway of ECM and inhibited the collagen synthetic pathway.

Transduction with the HGF gene also suppresses the increase of TGF-β1. Recently, Nakamura et al. [126] have shown that HGF is down-regulated in late-stage of disease in cardiomyopathic hamsters, which is related to severe cardiac dysfunction and fibrosis, accompanied by increases in myocardial expression of TGF-β1. Using the hypertrophied heart model of spontaneously hypertensive rats, Nakano et al. [127] showed that there were negative regulation of HGF by Ang II and TGF-β. Thus, the down-regulation of TGF-β1 by exogenous HGF may lead to therapeutic benefits in case of dilated cardiomyopathic hamster [126] and experimental models of post-infarction heart failure [128, 129].

Potential mechanisms for slowing progression of heart failure via regulating TGF-β

Each of the steps of synthesis, activation, and signaling of TGF-β represents a potential mechanism for regulating the activity of TGF-β (Figs 1, 3). While these mechanisms are primarily important for physiological regulation of TGF-β activity, there is increasing attention in using them to block overexpression of TGF-β-mediated tissue response to fibrogenic stimuli [11, 47].

Attempts to block the effects of excessive TGF-β activity have involved the use of neutralizing antibodies and natural TGF-β inhibitors (*e.g.* decorin), both of which inhibit TGF-β binding to its receptors. TGF-β1 knockout mice showed no hypertrophic responses to suppressor doses of Ang II [77].

Changes in Smad expression have been associated with heart diseases; for instance, the levels of Smad 2 and Smad 4 are up-regulated in scar tissue after MI [14]. This effect is attenuated by AT_1 receptor blocker, suggesting

the presence of cross-talk between Ang II and Smad signaling [130].

In a recent study, Yndestad et al. [103] have shown that level of activin A, a member of the TGF-β superfamily, is significantly elevated in patients with heart failure, suggesting the involvement of this cytokine in the pathogenesis of heart failure. Moreover, Li et al. [90] have demonstrated that cardiac TGF-β1 mRNA and protein are increased in samples obtained from patients with hypertrophic cardiomyopathy or aortic stenosis.

The TGF-β expression as a new target for therapy in progression of heart failure?

Several forms of antifibrotic therapy have recently emerged as possible new and promising treatment modalities in heart failure models in addition to conventional cardiovascular treatment regimens (Table 1).

Tranilast. Given the central role of TGF-β1 in myocardial fibrosis, as one of the hallmarks of myocardial remodeling in progression of heart failure [131], therapeutic modulation targeting this growth factor has received much attention. Ikeda et al. [132] demonstrated the inhibitory effect of tranilast on TGF-β-mediated collagen formation in an *in vitro* study. Recently, Pinto et al. [133] reported the effect of tranilast on reduction of TGF-β1 mRNA, suggesting the attenuation of myocardial fibrosis and improvement in survival in a hypertensive rat model. However, reduction in the expression of TGF-β1 did not prevent impairment of left ventricular function.

Anti-TGF-β1 neutralizing antibody. Using anti-TGF-β1 neutralizing antibody (NAbs), Koyanagi et al. [74] demonstrated the beneficial effects of this drug to prevent the infiltration of monocytes and myofibroblast formation in the heart and vessels of the rat model with chronic inhibition of NO synthesis. Tomita et al. [40] have shown that NAbs prevented an increase in the mRNA levels of ECM protein. Another study documented that anti-TGF-β1 antibody prevented myocardial fibrosis and diastolic dysfunction in pressure-overloaded rat, which may prevent further progression of heart failure and death [71].

Table 1. Antifibrotic treatment in progression of heart failure: potential treatment modalities.

Agent	References
Tranilast	[132, 133]
Anti-TGF-β1 neutralizing antibody (NAbs)	[40, 71, 74]
Endoglin antibody	[102]
Soluble TGF-β type II receptor	[138]
Anti-sense oligonucleotides	[140, 141]
Pirfenidone	[145–147]

Although there are only few published studies with tranilast and NAbs, and these are limited to experimental conditions, their consistent therapeutic successes make clear the enormous clinical potential associated with decreasing the action of TGF-β1 *in vivo*.

Endoglin antibody. Endoglin, a TGF-β binding protein [134], modulates the function of TGF-β1 [135]. Recent reports have shown that endoglin is expressed in stromal tissues, and may modulate fibrosis in progressive renal disease, scleroderma, and post-therapy breast fibrosis [134–136]. Moreover, Chen et al. [102] have shown that endoglin is expressed in cultured cardiac fibroblasts and mediates cardiac fibrosis via AT_1 receptor. Beneficial effects of endoglin antibody have been suggested through decreasing the expression of type I collagen protein and increasing collagen degrading enzyme MMP-1 in an *in vitro* study [104]. Thus, endoglin antibody may provide a potential therapy for Ang II-induced TGF-β1 by modulating cardiac fibroblast function. However, further data are needed to identify more precisely the role of endoglin in post-infarction heart failure.

Soluble TGF-β type II receptor. Soluble TGF-β type II receptor (sTβRII) inhibits the action of TGF-β, most likely by adsorbing TGF-β or by acting as a dominant negative receptor [137]. Using a mouse model of coronary ligation, Okada et al. [138] have recently shown that inhibition of circulating TGF-β1 through adenoviral-mediated overexpression of sTβRII attenuated post-MI fibrosis and infarct wall thinning, and decreased ventricular chamber dilation, improving post-infarct contractile function and mortality. Treatment with adenovirus expressing sTβRII at 3 days post-infarct resulted in a beneficial remodeling response, whereas treatment initiated at 4 weeks was ineffective. Such observations indicate that TGF-β1 may be protective at the time of myocardial ischemia, although deleterious soon thereafter because of acute remodeling [139].

TGF-β antisense oligonucleotides. As the complexities of TGF-β regulation are unraveled, manipulation of its regulation may lead to decreased production of TGF-β [11]. Inhibition of gene expression by antisense oligodeoxynucleotides relies on their ability to bind complementary mRNA sequences and prevent translation [140, 141]. Using the TGF-β-producing C3H/He-MBT-2 murine bladder tumor model, Tsai et al. [142] demonstrated that reducing the secretion of TGF-β1 on tumor cells by TGF-β1 antisense oligonucleotide could inhibit their *in vitro* growth and *in vivo* tumor formation, suggesting that this approach can be a potentially useful strategy to abolish the adverse immunosuppression effect of TGF-β1-producing autologous tumor vaccine. Furthermore, Ihn et al. [143] showed that the blockade of TGF β sig-

naling with a TGF-β1 antisense oligonucleotide abolished the increased mRNA expression in scleroderma fibroblasts.

Recent studies in humans and experimental models have shown increased myocardial TGF-β expression during cardiac hypertrophy and fibrosis [40, 77]. Antisense technologies can be expected to be widely used for studies of genes with unknown function, for target validation in drug development and finally, of course, for therapeutic purpose [144]. Thus, using TGF-β antisense oligonucleotides is another possibility to prevent cardiac fibrosis.

Pirfenidone. Pirfenidone, an antifibrotic agent, is known to inhibit progression of fibrosis in animal models and idiopathic pulmonary fibrosis patients [145–147]. Moreover, Iyer and coworkers [148] demonstrated that treatment with pirfenidone suppresses the bleomycin-induced increases in the TGF-β levels of the bleomycin hamster model of lung fibrosis. This effect finally led to a reduction in the synthesis and deposition of collagen in the lung of hamsters. However, whether the beneficial effects of pirfenidone would also apply to cardiac fibrosis needs further investigation.

Conclusions

Experimental and clinical studies have conclusively demonstrated that TGF-β plays a critical role in the progression of cardiac remodeling, a key determinant of the clinical course of heart failure. TGF-β1 is expressed in MI, pressure-overload, cardiomyopathies, and with Ang II and norepinephrine infusions. Ang II induces the expression of TGF-β1 in cardiomyocytes and fibroblasts. Increased expression of TGF-β1 results in increased production and decreased degradation of extracellular collagen matrix, and play a key role in mediating cardiac hypertrophy and fibrosis. Antagonists of TGF-β1 may play an important role in preventing progression of cardiac remodeling in heart failure. However, TGF-β signaling involves extensive cross-talk with other signaling pathways. The complexity of these interactions is only beginning to be defined. These interacting pathways may lead to more specific agents designed to inhibit myocardial fibrosis triggered by TGF-β1 in the progression of heart failure. A number of possible therapeutic approaches for decreasing the action of TGF-β1 have been suggested, such as tranilast, NAbs, endoglin antibody, soluble TGF-β type II receptor, TGF-β antisense oligonucleotide, and pirfenidone. Whether any of these approaches will yield an affective antifibrotic drug is unknown. However, understanding that TGF-β1 is a key factor in myocardial fibrosis offers a target for new therapy in the progression of heart failure.

1 Cohn, J. N., Bristow, M., Chien, K. R., Colucci, W. S., Frazier, O. H., Leslie, A., Leinwand, L. A., Lorell, B. H., Moss, A. J., Sonnenblick, E. H, Walsh, R. A., Mockrin, S. C. and Reinlib, L. (1997) Report of the National Heart, Lung, and Blood Institute special emphasis panel on heart failure research. Circulation 95, 766–770.

2 Hunt, S. A., Baker, D. W., Chin, M. H., Cinquergrani, M. P., Feldman, A. M., Francis, G. S., Ganiats, T. G., Glodstein, S., Gregoratos, G., Jessup, M. L., Noble, R. J., Packer, M., Silver, M. A., Stevenson, L. W., Gibbons, R. J., Antman, E. M., Alpert, J. S., Faxon, D. P., Fuster, V., Jacobs, A. K., Hiratzka, L. F., Russell, R. O. and Smith, S. C. (2001) ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult. J. Am. Coll. Cardiol. 38, 2101–2113.

3 Jugdutt, B. (2003) Ventricular remodeling after infarction and the extracellular collagen matrix. When enough is enough ? Circulation 108, 1395–1403.

4 Yan, A. T., Yan, A. R. and Liu, P. P. (2005) Pharmacotherapy for chronic heart failure: evidence from recent clinical trials. Ann. Intern. Med. 142, 132–145.

5 Ju, H., Zhao, S., Tappia, P. S., Panagia, V. and Dixon, I. M. C. (1998) Expression of $G_{q\alpha}$ and PLC-β in scar and border tissue in heart failure due to myocardial infarction. Circulation 97, 892–899.

6 Pfeffer, J. M., Fischer, T. A. and Pfeffer, M. A. (1995) Angiotensin-converting enzyme inhibition and ventricular remodeling after myocardial infarction. Annu. Rev. Physiol. 57, 805–826.

7 Sutton, M. G. S. J. and Sharpe, N. (2000) Left ventricular remodeling after myocardial infarction. Pathophysiology and therapy. Circulation 101, 2981–2988.

8 Peterson, J. T., Hallak, H., Johnson, L., Li Hua., O'Brien, P. M., Sliskovic, D. R., Bocan, T. M. A., Coker, M. L., Etoh, T. and Spinale, F. G. (2001) Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. Circulation 103, 2303–2309.

9 Cohn, J. N., Ferrari, R. and Sharpe, N. (2000) Cardiac remodeling – concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an international forum on cardiac remodeling. J. Am. Coll. Cardiol. 35, 569–582.

10 Kim, H. E., Dala, S. S., Young, E., Legato, M. L., Weisfeldt, M. L. and D'Armiento, J. (2000) Disruption of the myocardial extracellular matrix leads to cardiac dysfunction. J. Clin. Invest. 106, 857–866.

11 Border, W. A. and Noble, N. A. (1994) Transforming growth factor β in tissue fibrosis. N. Engl. J. Med. 331, 1286–1292.

12 Ju, H., Zhao, S., Tappia, P. S., Panagia, V. and Dixon, I. M. C. (1997) Effects of AT_1 receptor blockade on cardiac collagen remodeling after myocardial infarction. Cardiovasc. Res. 35, 223–232.

13 Weber, K. T. (1997) Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. Circulation 96, 4065–4082.

14 Hao, J., Wang, B., Jones, S. C., Jassal, D. S. and Dixon, I. M. C. (2000) Interaction between angiotensin II and Smad proteins in fibroblasts in failing heart and *in vivo*. Am. J. Physiol. Heart Circ. Physiol. 279, H3020–H3030.

15 Bartram, U. and Speer, C. P. (2004) The role of transforming growth factor β in lung development and disease. Chest 125, 754–765.

16 Cate, R. L., Mattaliano, R. J., Hession, C., Tizard, R., Farber, N. M., Cheung, A., Ninfa, E. G., Frey, A. Z., Gash, D. J., Chow, E. D., Fisher, R. A., Bertonis, J. M., Torres, G., Wallner, B. D., Ramachandran, K. L., Ragin, R. C., Manganaro, T. F., MacLaughlin, D. T. and Donahoe, P. K. (1986) Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. Cell 45, 685–698.

17 Smith, J. C. (1993) Mesoderm-inducing factors in early vertebrate development. EMBO J. 12, 4463–4470.
18 Wozney, J. M., Rosen, V., Celeste, A. J., Mitsock, L. M., Whitters, M. J., Kriz, R. W. and Wang, E. A. (1988) Novel regulators of bone formation: molecular clones and activities. Science 242, 1528–1534.
19 Pelton, R. W., Saxena, B., Jones, M., Moses, H. L. and Gold, L. I. (1991) Immunohistochemical localization of TGF β1, TGF β2, and TGF β3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. J. Cell Biol. 115, 1091–1105.
20 Fujii, D., Brissenden, J. E., Derynck, R. and Francke, U. (1986) Transforming growth factor β genes maps to human chromosome 19 long arm and to mouse chromosome 7. Somat. Cell Mol. Genet. 12, 281–288.
21 Barton, D. E., Foellmer, B. E. and Du, J. (1988). Chromosomal mapping of genes for transforming growth factors β 2 and β 3 in man and mouse: dispersion of TGF-β gene family. Oncogene Res. 3, 323–331.
22 Wells, R. G. (2000) Fibrogenesis, V. TGF-β signaling pathways. Am. J. Physiol. Gastrointest. Liver Physiol. 279, G845–G850.
23 Grande, J. P. (1997) Role of transforming growth factor-beta in tissue injury and repair. Exp. Biol. Med. 214, 27–40.
24 Cheng, J. and Grande, J. P. (2002) Transforming growth factor-β signal transduction and progressive renal disease. Exp. Biol. Med. 227, 943–956.
25 Blobe, G. C., Schiemann, W. P. and Lodish, H. F. (2000) Role of transforming growth factor-β in human disease. N. Engl. J. Med. 18, 1350–1358.
26 Goumans, M. J. and Mummery, C. (2000). Functional analysis of the TGF beta receptor/Smad pathway through gene ablation in mice. Int. J. Dev. Biol. 44, 253–265.
27 Kobayashi S, Yoshida K, Ward J, Letterio J, Longenecker, G., Yaswen, L., Mittleman, B., Mozes, E., Roberts, A., Karlsson, S. and Kulkarni, A. (1999) β_2-microglobulin-deficient background ameliorates lethal phenotype of the TGF-β1 null mouse. J. Immunol. 163, 4013–4019.
28 Shull, M. M., Ormsby, I. Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., Annunziata, N. and Doetschman, T. (1992) Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. Nature 359, 693–699.
29 Kulkarni, A. B., Huh, C. G., Becker, D., Geiser, A., Lyght, M., Flanders, K. C., Roberts, A. B., Sporn, M. B., Ward, J. M. and Karlsson, S. (1993) Transforming growth factor β1 null mutation in mice causes excessive inflammatory response and early death. Proc. Natl. Acad. Sci. USA 90, 770–774.
30 Sanford, L. P, Ormsby, I., Gittenberger-de Groot, A. C., Sariola, H., Friedman, R., Boivin, G. P., Cardell, E. L. and Doetschman, T. (1997) TGF-β2 knockout mice have multiple developmental defects that are non-overlapping with other TGF-β knockout phenotypes. Development 124, 2659–2670.
31 Bartram, U., Molin, D. G., Wisse, L. J, Mohamad, A., Sanford, L. P., Doetschman, T., Speer, C. P., Poelmann, R. E. and Gittenberger-de Groot, A. C. (2001) Double-outlet right ventricle and overriding tricuspid valve reflect disturbances of looping, myocardialization, endocardial cushion differentiation, and apoptosis in TGF β (2)-knockout mice. Circulation 103, 2745–2745.
32 Kaartinen V, Voncken, J. W, Shuler C, Warburton D, Bu D, Heisterkamp, N., Groffen, J. (1995) Abnormal lung development and cleft palate in mice lacking TGF-β3 indicates defects of epithelial-mesenchymal interaction. Nat. Genet. 11, 415–421.
33 Branton, M. H. and Kopp, J. B. (1999) TGF-β and fibrosis. Microbes Infect. 1, 1349–1365.
34 Gentry, L. E., Webb, N. R., Lim, G. J., Brunner, A. M., Ranchalis, J. E., Twardzik, D. R. Lioubin, M. N, Marquardt, H. and Purchio, A. F. (1987) Type 1 transforming growth factor β: amplified expression and secretion of mature and precursor polypeptides in Chinese hamster ovary cells. Mol. Cell. Biol. 7, 3418–3427.
35 Harpel, J. G., Metz, C. N., Kojima, S. and Rifkin, D. B. (1992) Control of transforming growth factor-β activity: latency *vs*. activation. Prog. Growth Factor Res. 4, 321–335.
36 Gleizes, P. E., Munger, J. S., Nunes I, Harpel, J. G., Mazzieri, R. and Noguera, I. (1997) TGF-β latency: biological significance and mechanisms of activation. Stem Cells 15, 190–197.
37 Annes, J. P., Munger, J. S. and Rifkin, D. B. (2003) Making sense of latent TGF-β activation. J. Cell Sci. 116, 217–224.
38 Schultz-Cherry, S., Ribeiro, S., Gentry, L. and Murphy-Ullrich, J. E. (1994) Thrombospondin binds and activates the small and large forms of latent transforming growth factor-β in a chemically defined system. J. Biol. Chem. 269, 26775–26782.
39 Lyons, R. M., Keski-Oja, J. and Moses, H. L. (1988) Proteolytic activation of latent transforming growth factor β from fibroblast-conditioned medium. J. Cell Biol. 106, 1659–1665.
40 Tomita, H., Egashira, K. and Ohara, Y. (1998) Early induction of transforming growth factor-β via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. Hypertension 32, 273–279.
41 Fortunel, N. O., Hatzfeld, A. and Hatzfeld, J. A. (2000) Transforming growth factor-β: pleiotropic role in the regulation of hematopoiesis. Blood 96, 2022–2030.
42 Massaque, J. (1998) TGF-β signal transduction. Annu. Rev. Biochem. 67, 753–791.
43 Kretzschmer, M. and Massague, J. (1998) SMADs: mediators and regulation of TGF-beta signaling. Curr. Opin. Genet. Dev. 8, 103–111.
44 Massague J, Seoane J, Wotton, D. (2005) Smad transcription factors. Genes Dev. 19, 2783–2810.
45 Dumans, M. J, Gouman, M. J and ten Dijke, P. (2002) Transforming growth factor β signal transduction. J. Leukoc. Biol. 71, 731–740.
46 Prime, S. S., Pring, M., Davies, M. and Paterson, I. C. (2004) TGF-β signal transduction in oro-facial health and non-malignant disease (Part I). Crit. Rev. Oral Biol. Med. 15, 324–336.
47 Janssens K, ten Dijke P, Janssens, S. and Hul, W. V. (2005) Transforming growth factor-β1 to the bone. Endocr. Rev. 26, 743–774.
48 Massague, J. and Chen, Y. G. (2000) Controlling TGF-signaling. Genes Dev. 4, 627–644.
49 Javelaud, D. and Mauviel, A. (2004) Mammalian transforming growth factor-betas:Smad signaling and physiopathological roles. Int. J. Biochem. Cell Biol. 36, 1161–1165.
50 Labbe, E., Silvestri, C., Hoodless, P. A., Wrnas, J. L. and Attisano, L. (1998) Smad 2 and Smad 3 positively and negatively regulate TGF-beta-dependent transcription through the forhead DNA-binding protein FAST2. J. Mol. Cell. Cardiol. 2, 109–120.
51 Wang, B., Hao, J., Jones, S. C., Yee, M. S., Roth, J. C. and Dixon, I. M. C. (2002) Decreased Smad 7 expression contributes to cardiac fibrosis in the infarcted rat heart. Am. J. Physiol. Heart Circ. Physiol. 282, 1685–1696.
52 Euler-Taimor G, Heger, J. (2006) The complex pattern of SMAD signaling in the cardiovascular system. Cardiovasc. Res. 69, 15–25.
53 Shi, Y., Wang, Y. F., Jayaraman, L., Yang, H., Massague, J. and Pavletich, N. P. (1998) Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-β signaling. Cell 94, 585–594.
54 Wu, R., Zhang, Y., Feng, X. and Derynck, R. (1997) Heteromeric and homomeric interactions correlate with signaling activity and functional cooperativity of Smad3 and Smad 4/DPC4. Mol. Cell. Biol. 17, 2521–2528.

55 Zhang. Y, Feng, X. H., Derynck, R. (1998) Smad 3 and Smad 4 cooperate with c-Jun/c-Fos to mediate TGF-beta-induced transcription. Nature 394, 909–913.

56 Wong, C., Rougier-Chapman, E. M., Frederick, J. P., Datto, M. B., Leberati, N. T., Li, J. M. and Wang, X. F. (1999) Smad3-Smad 4 and AP-1 complexes synergize in transcriptional activation of the c-Jun promoter by transforming growth factor-beta. Mol. Cell. Biol. 19, 1812–1830.

57 Rodriguez-Pascual, F., Redondo-Horcajo, M. and Lamas, S. (2003) Functional cooperation between Smad proteins and activator protein-1 regulates transforming growth factor-mediated induction of endothelin-1 expression. Circ. Res. 92, 1288–1295.

58 Shibuya, H., Yamaguchi, K., Shirakabe, K., Tenegawa, A., Gotoh, Y. and Ueno, N. (1996) TAB1: an activator of the TAK1 MAPKKK in TGF-β signal transduction. Science 272, 1179–1182.

59 Moriguchi, T., Karoyanagi, N., Yamaguchi, K., Gotoh, Y., Irie, K., Kano, T., Shirakabe, K., Muro, Y., Shibuya, H., Matsumoto, K., Nishida, E. and Hagiwara, M. (1996) A novel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. J Biol. Chem. 27, 13675–13679.

60 Sano, Y., Harada, J., Tashiro, S., Gotoh-Mandeville, R., Maekawa, T. and Ishii, S. (1999) ATF-2 is a common nuclear target of smad and TAK1 pathways in transforming growth factor-β signaling. J. Biol. Chem. 274, 8949–8957.

61 Schulz, R., Aker, S., Belosjorow, S., Konietzka, I., Rauen, U. and Heusch, G. (2003) Stress kinase phosphorylation is increased in pacing-induced heart failure in rabbits. Am. J. Physiol. Heart Circ. Physiol. 285, 2084–2090.

62 Cook, S. A., Sugden, P. H. and Clerk, A. (1999) Activation of c-Jun N-terminal kinases and p38-mitogen-activated protein kinases in human heart failure secondary to ischaemic heart disease. J. Mol. Cell. Cardiol. 31, 1429–1434.

63 Court, N. W., dos Remedios, C. G., Cordell, J. and Bogoyevitch, M. A. (2002) Cardiac expression and subcellular localization of the p38 mitogen-activated protein kinase member, stress-activated protein kinase-3 (SAPK3). J. Mol. Cell. Cardiol. 34, 413–426.

64 Kawai, H., Stevens, S. Y. and Liang, C. S. (2000) Renin-angiotensin system inhibition on noradrenergic nerve terminal function in pacing-induced heart failure. Am. J. Physiol. Heart Circ. Physiol. 279, H3012–H3030.

65 Packer, M. (1990) Role of the sympathetic nervous system in chronic heart failure. Circulation 82, Suppl I, I-1–I-6.

66 Qin, F., Shite, J. and Liang, C. (2003) Antioxidants attenuates myocyte apoptosis and improve cardiac function in heart failure: association with changes in MAPK pathways. Am. J. Physiol. Heart Circ. Physiol. 285, H822–H832.

67 Haq, S., Choukroun, G., Lim, H., Tymitz, K. M., del Monte, F., Gwathmey, J., Grazette, L., Michael, A., Hajjer, R., Force, T. and Molkentin, J. D. (2001) Differential activation of signal transduction pathways in human hearts with hypertrophy versus advanced heart failure. Circulation 103, 670–677.

68 Matsumoto-Ida, M., Takimoto, Y., Aoyama, T., Akao, M., Takeda, T. and Kita, T. (2006) Activation of TGF-β1-TAK1-p38 MAPK pathway in spared cardiomyocytes is involved in left ventricular remodeling after myocardial infarction in rats. Am. J. Physiol. Heart Circ. Physiol. 290, H709–H715.

69 See, F., Thomas, W., Way, K., Tzanidis, A., Kompa, A. and Lewis, D. (2004) p38 mitogen-activated protein kinase inhibition improves cardiac function and attenuates left ventricular remodeling following myocardial infarction in the rat. J. Am. Coll. Cardiol. 44, 1679–1689.

70 Sun, Y. and Weber, K. T. (2000) Infarct scar: a dynamic tissue. Cardiovasc. Res. 46, 250–256.

71 Kuwahara, F., Kai, H., Tokuda, K., Kai, M., Takeshita, A. and Egashira, K. (2002) Transforming growth factor-β function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. Circulation 106, 130–135.

72 Sun, Y., Zhang, J. Q., Zhang, J. and Ramires, F. J. (1998) Angiotensin II, transforming growth factor-beta1 and repair in the infarcted heart. J. Mol. Cell. Cardiol. 8, 1559–1569.

73 Takahashi, N., Calderone, A., Izzo, N. J., Maki, T. M., Marsh, J. D. and Colucci, W. (1994) Hypertrophic stimuli induce transforming growth factor-beta 1 expression in rat ventricular myocytes. J. Clin. Invest. 94, 1470–1476.

74 Koyanagi, M., Egashira, K., Kubo-Inoue, M., Usui, M., Kitamoto, S. and Tomita H, (2000) Role of transforming growth factor-β1 in cardiovascular inflammatory changes induced by chronic inhibition of nitric oxide synthesis. Hypertension 35, 86–90.

75 Eghbali, M. (1989) Cellular origin and distribution of transforming growth factor-beta in the normal rat myocardium. Cell Tissue Res. 256, 553–558.

76 Thompson, N. L. (1988) Transforming growth factor-beta 1 in acute myocardial infarction in rats. Growth factors 1, 91–99.

77 Villareal, F. J. and Dillmann, W. H. (1992) Cardiac hypertrophy-induced changes in mRNA levels for TGF-beta 1, fibronectin, and collagen. Am. J. Physiol. 262, H1861–H1866.

78 Kupfahl, C., Pink, D., Friedrich, K., Zurbrugg, H. R., Neuss, M., Warnecke, C., Fielitz, J., Graf, K., Fleck, E. and Regitz-Zagrosek, V. (2000) Angiotensin II directly increases transforming growth factor beta 1 and osteopontin and indirectly affects collagen mRNA expression in the human heart. Cardiovasc. Res. 46, 463–475.

79 Sun, Y., Cleutjen, J. P. M., Diaz-Arias, A. A. and Weber, K. T. (1994) Cardiac angiotensin-converting enzyme and myocardial fibrosis in the rat. Cardiovasc. Res. 28, 1423–1432.

80 Smits, J. F. M, van Krimpen, C., Schoemaker, R. G., Cleutjen, J. P. M and Daemen, M. J. A. P. (1992) Angiotensin II receptor blockade after myocardial infarction in rats: effects on haemodynamics, myocardial DNA synthesis, and interstitial collagen content. J. Cardiovasc. Pharmacol. 20, 772–778.

81 Volders, P. G. A., Willems, I. E. M. G., Cleutjens, J. P. M., Arends J.-W., Havenith, M. G. and Daemen, M. J. A. P. (1993) Interstitial collagen is increased in the non-infarcted human myocardium after myocardial infarction. J. Mol. Cell. Cardiol. 25, 1317–1323.

82 van Krimpen C,. Smits, J. F. M., Clutjens, J. P. M., Debetd, J. J., Schoemake, R. G., Struyker-Boudier, H. A., Bosman, F. T. and Daemen, M. J. A. P. (1991) DNA synthesis in the non-infarcted cardiac interstitium after coronary artery ligation in the rat heart: effects of captopril. J. Mol. Cell. Cardiol. 23, 1245–1253.

83 Parker, T. G., Packer, S. E, and Schneider, M. D. (1990) Peptide growth factors can provoke 'fetal' contractile protein gene expression in rat cardiac myocytes. J. Clin. Invest. 85, 507–514.

84 Schultz, J. E., Witt, S. A., Glascock, B. J., Nieman, M. L., Reiser, P. J. Nix, S. L., Kimball, T. R. and Doetschman, T. (2002) TGF-β1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. J. Clin. Invest. 109, 787–796.

85 Chapman, D., Webet, K. T. and Egbali, M. (1990) Regulation of fibrillar collagen types I and III and basement membrane type IV collagen gene expression in pressure overload rat myocardium. Circ. Res. 67, 787–794.

86 Bhambi, B. and Eghbali, M. (1991) Cardiac hypertrophy-induced changes gene expression and response of cardiac fibroblasts after norepinephrine treatment. Am. J. Pathol. 139, 1131–1142.

87 Rosenkranz, S., Flesch, M., Amann, K., Haeuseler, C., Kilter, H., Seeland, U., Schluter, K. D. and Bohm, M. (2002) Alterations of β-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF-β_1. Am. J. Physiol. Heart Circ. Physiol. 283, H1253–H1262.

88 Pauschinger, M., Knopf, D., Petschauer, S., Doerner, A., Poller, W. and Schwimmbeck, P. L. (1999) Dilated cardiomyopathy is associated with significant changes in collagen type I/III ratio. Circulation 99, 2750–2756.

89 Sanderson E, Lai, K. B., Shum, I. O. L., Wei, S. and Chow, L. T. C. (2001) Transforming growth factor-beta 1 expression in dilated cardiomyopathy. Heart 86, 701–708.

90 Li, R. K., Li, G. M., Meckle, D. A. G., Weisel, R. D., Merante, F., Luss, H., Rau, V., Christakis, G. T. and Williams, W. G. (1997) Overexpression of transforming growth factor-β1 and insulin-like growth factor 1 in patients with idiopathic hypertrophic cardiomyopathy. Circulation 96, 874–881.

91 Wang, B., Hao, J., Jones, S. C., Yee, M. S., Roth, J. C., Dixon, I. M. C. (2002) Decreased Smad 7 expression contributes to cardiac fibrosis in the infarcted rat heart. Am. J. Physiol. Heart Circ. Physiol. 282, H 1685–H 1696.

92 Zhu, Y. Z. and Huang, S. H. (2000) Cardioprotective actions of angiotensin-converting enzyme inhibitors after myocardial infarction. Cor. Europaeum 8, 154–160.

93 Guerrero-Esteo, M., Sanchez-Elsner, T., Letamendia, A. and Bernabeu, C. (2002) Extracellular and cytoplasmic domains of endoglin interact with the transforming growth factor-beta receptors I and II. J. Biol. Chem. 291, 197–209.

94 Gray, M. O., Long, S. C., Kalinyak, J. E., Li, H. T. and Karliner, J. S. (1998) Angiotensin II stimulates cardiac myocytes hypertrophy via paracrine release of TGF-beta 1 and endothelin-1 from fibroblasts. Cardiovasc. Res. 40, 352–363.

95 Sun, Y., Zhang, J. Q., Zhang, J. and Ramires, F. J. (1998) Angiotensin II, transforming growth factor-beta 1 and repair in the infarcted heart. J. Mol. Cell. Cardiol. 30, 1559–1569.

96 Yue, P., Massie, B. M., Simpson, P. C. and Long, C. S. (1998) Cytokine expression increases in nonmyocytes from rats with postinfarction heart failure. Am. J. Physiol. Heart Circ. Physiol. 44, H250–H258.

97 Weber, K. T. (1989) Cardiac interstitium in health and disease: the fibrillar collagen network. J. Am. Coll. Cardiol. 13, 1673–1652.

98 Lijnen, P., Petrov, V. V. and Fagard, R. (2000) Induction of cardiac fibrosis by transforming growth factor beta (1). Mol. Genet. Metab. 71, 418–435.

99 Hao, J., Ju, H., Zhao, S., Junaid, A., Scammell-LaFleur, T. K. and Dixon, I. M. C. (1999) Elevation of expression of Smad 2, 3, and 4, decorin and TGF-beta in the chronic phase of myocardial infarct scar healing. J. Mol. Cell. Cardiol. 31, 667–678.

100 Koyanagi, M., Egashira, K., Kubo-Inoue, M., Usui M, Kitamoto, S., Tomita, H., Shimokawa, H. and Takeshita, A., (2000) Role of transforming growth factor-β1 in cardiovascular inflammatory changes induced by chronic inhibition of nitric oxide synthesis. Hypertension 35, 86–90.

101 Weber, K. T. and Brilla, C. G. (1991) Pathological hypertrophy and cardiac interstitium: fibrosis and the renin-angiotensin aldosterone system. Circulation 83, 1849–1865.

102 Chen, K., Mehta, J. L., Li, D., Joseph, L. and Joseph, J. (2004) Transforming growth factor-β receptor endoglin is expressed in cardiac fibroblasts and modulates profibrogenic actions of angiotensin II. Circ. Res. 95, 1167–1173.

103 Yndestad, A., Ueland, T., Oie, E., Florholmen, G., Halvorsen, B. and Attramadal, H. (2004) Elevated levels of activin A in heart failure. Potential role in myocardial remodeling. Circulation 109, 1379–1385.

104 Peng, J., Gurantz, D., Tran, V., Cowling, R. T. and Greenberg, B. H. (2002) Tumor necrosis factor-alpha induced AT_1 receptor upregulation enhances angiotensin II-mediated cardiac fibroblast responses that favor fibrosis. Circ. Res. 91, 1119–1126.

105 Deten, A., Voltz, H. C., Briest, W. and Zimmer, H. G. (2002) Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats. Cardiovasc. Res. 55, 329–340.

106 Irwin, M., Mak, S., Mann, D., Qu, R., Dawood, F., Wen, W. H. et al. (1999) Tissue expression and immunolocalization of tumor necrosis factor-alpha post infarction-dysfunctional myocardium. Circulation 99, 1492–1498.

107 Nian, M., Lee, P., Khaper, N. and Liu, P. (2004) Inflammatory cytokines and postmyocardial infarction remodeling. Circ. Res. 94, 1543–1553.

108 Werner, S. and Grose, R. (2002) Regulation of wound healing by growth factors and cytokines. Physiol. Rev. 83, 835–870.

109 Rodríguez-Vita, J., Sánchez-López, E., Esteban, V., Rupérez, M., Egido, J. and Ruiz-Ortega, M. (2005) Angiotensin II activates the smad pathway in vascular smooth muscle cells by a transforming growth factor β-independent mechanism. Circulation 111, 2509–2517.

110 Border, W. A. and Ruoslahti, E. (1992) Transforming growth-factor in disease: the dark side of tissue repair. J. Clin. Invest. 1, 1–7.

111 Rosenkranz, S. (2004) TGF-β1 and angiotensin networking in cardiac remodeling. Cardiovasc. Res. 63, 423–432.

112 Wenzel S, Taimor G, Piper, H. M, Schafer, K. D. (2001) Redox-sensitive intermediates mediate angitoensin II-induced p38 MAP kinase activation, AP-1 binding activity, and TGF-β1 expression in adult ventricular cardiomyocytes. FASEB J. 15, 2291–2293.

113 Chen, M. M., Lam, A., Abraham, J. A., Schreiner, G. E. and Jul, A. H. (2000) CTGF expression is induced by TGF-beta in cardiac fibroblasts and cardiac myocytes: a potential role in heart fibrosis. J. Mol. Cell. Cardiol. 32, 1805–1819.

114 Ruperez, M., Lorenzo, O., Blanco-Colio, M. B., Esteban, V. Egido, J. and Ruiz-Ortega, M. (2003) Connective tissue growth factor is mediator of angiotensin II-induced fibrosis. Circulation 108, 1499–1505.

115 Abraham, D. J., Xu, S. W., Black, C. M., Sa. S., XuY. I. and Leask, A. (2000) Tumor necrosis factor-α suppresses the induction of connective tissue growth factor by transforming growth factor-β in normal and scleroderma fibroblasts. J. Biol. Chem. 275, 15220–15225.

116 Grotendorst, G. R., Okochi, H. and Hayashi, N. (1996) A novel transforming growth factor beta response element controls the expression of the connective tissue growth factor gene. Cell Growth Differ. 7, 469–480.

117 Leask, A., Holmes, A., Black, H. C. and Abraham, D. J. (2003) Connective tissue growth factor gene regulation. J. Biol. Chem. 278, 13008–13015.

118 Duncan, M. R., Frazier, K. S., Abramson, S., Williams, S., Klapper, H., Huang, X. F. and Grotendorst, G. R. (1999) Connective tissue growth factor mediates transforming growth factor β-induced collagen synthesis: down-regulated by cAMP. FASEB J. 13, 1774–1786.

119 Lau, L. F. and Lam, S. C. T. (1999) The CCN family of angiogenic regulators: the integrin connection. Exp. Cell Res. 248, 44–57.

120 Kucich, U., Resonbloom, J. C., Herrick, D. J., Abrams, W. R., Hamilton, A. D, Sebti, S. M. and Rosenbloom, J. (2001) Signaling events required for transforming growth factor-beta stimulation of connective tissue growth factor expression by cultured human lung fibroblasts. Arch. Biochem. Biophys. 395, 103–112.

121 Ueki, T., Kaneda, Y., Tsutsui, H., Nakanushi, K., Sawa, Y. and Morishita, R. (1999) Hepatocyte growth factor gene therapy of liver cirrhosis in rats. Nat. Med. 5, 226–230.

122 Nakamura, T., Nishizawa Tsutomu., Hagiya, M., Seki, T., Shimonishi M, Sugimura, A., Tashiro, K. and Shimizu, S. (1989) Molecular cloning and expression of human hepatocyte growth factor. Nature 342, 440–443.

123 Taniyama, Y., Morishita, R., Nakagami, H., Moriguchi, A., Sakonjo, H., Kim, S., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (2000) Potential contribution of a novel antifibrotic factor, hepatocyte growth factor, to prevention of myo-

cardial fibrosis by angiotensin II blockade in cardiomyopathic chamsters. Circulation 102, 246–252.
124 Matsuda, Y., Matsumoto, K., Yamada, A., Ichida, T., Asakura, H., Komoriya, Y. et al. (1995) Hepatocyte growth factor suppresses the onset of liver cirrhosis and abrogates lethal hepatic dysfunction in rats. J. Biochem. (Tokyo) 118, 643–649.
125 Yaekashiwa, M., Nakayama, S., Ohnuma, K., Sakai, T., Abe, T., Satoh, K. et al. (1997) Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin: a morphologic study. J. Respir. Crit. Care Med. 156, 1937–1944.
126 Nakamura, T., Matsumoto, K., Mizuno, S., Sawa, Y., Matsuda, H. and Nakamura, T. (2005) Hepatocyte growth factor prevents tissue fibrosis, remodeling, and dysfunction in cardiomyopathic hamster hearts. Am. J. Physiol. Heart Circ. Physiol. 288, H2131–H2139.
127 Nakano, N., Morishita, R., Moriguchi, A., Nakamura, Y., Hayashi, S., Aoki, M., Kida, I., Matsumoto, K., Nakamura, T., Higaki, J. and Ogihara, T. (1998) Negative regulation of local hepatocyte growth factor expression by angiotensin II and transforming growth factor-β in blood vessels: potential role of HGF in cardiovascular disease. Hypertension 32, 444–451.
128 Jayasankar, V., Woo, J., Bish, L. T., Pirolli, T. J., Chatterjee, S., Berry, M. F. Burdick, J., Gardner, T. J. and Sweeney, L. (2003) Gene transfer of hepatocyte growth factor attenuates postinfarction heart failure. Circulation 108 [Suppl II], II-230–II-236.
129 Li, Y. W., Takemura, G., Kosai, K., Yage, K., Nagano. S., Esaki, M., Goto, K., Takahashi, T., Hayakawa, K., Koda, M., Kawase, Y., Maruyama, R., Okada, H., Minatoguchi, S., Mizuguchi, H., Fujiwara, T. and Fujiwara, H. (2003) Postinfarction treatment with an adenoviral vector expressing hepatocyte growth factor relieves chronic left ventricular remodeling and dysfunction in mice. Circulation 107, 2499–2506.
130 Manabe, I., Shindo, T. and Nagai, R. (2002) Gene expression in fibroblasts and fibrosis. Involvement in cardiac hypertrophy. Circ. Res. 91, 1103–1113.
131 Jessup, M. and Brozena, S. (2003) Heart failure. N. Engl. J. Med. 348, 2007–2018.
132 Ikeda, H., Inao, M. and Fujiwara, K. (1996) Inhibitory effect of tranilast on activation and transforming growth factor beta 1 expression in cultures rat stellate cells. Biochem. Biophys. Res. Commun. 227, 322–327.
133 Pinto, Y. M., Pinto-Sietsma, S. J., Philipp, T., Engler, S., Kossmehl, P., Hocher, B., Marquardt, H., Sethmann, S., Lauser, R., Merker H. J. and Paul, M. (2000) Reduction in left ventricular messenger RNA for transforming growth factor β1 attenuates left ventricular fibrosis and improves survival without lowering blood pressure in the hypertensive TGR (mRen2) 27 rat. Hypertension 36, 747–754.
134 Rodriguez-Pena, A., Prieto, M., Duwel, A., Rivas, J. V., Eleno, N., Perez-Barriocanal, F., Arevalo, M., Smith, J. D., Vary C. P., Bernabeu, C. and Lopez-Novoa, J. M. (2001) Up-regulation of endoglin, a TGF-beta-binding protein, in rats with experimental renal fibrosis induced by renal mass reduction. Nephrol. Dial. Transplant. 16 Suppl 1, 34–39.
135 Dharmapatni, A. A., Smith, M. D., Ahern, M. J., Simpson, A., Li, C., Kumar, S. and Roberts-Thomson, P. J. (2001) The TGF beta receptor endoglin in systemic sclerosis. Asian Pac. J. Allergy Immunol. 19, 275–282.
136 Li, C., Wilson, P. B., Levine, E., Stewart, A. L. and Kumar, S. (1999) TGF-beta1 levels in pre-treatment plasma identify breast cancer patients at risk of developing post-radiotherapy fibrosis. Int. J. Cancer. 84, 155–159.
137 Isaka, Y., Akagi, Y., Ando, Y., Tsujie, M., Sudo, T., Ohno, N., Border, W. A., Noble, N. A., Kaneda, Y., Hori, M. and Imai, E. (1999) Gene therapy by transforming growth factor-beta receptor-IgG Fc chimera suppressed extracellular matrix accumulation in experimental glomerulonephritis. Kidney Int. 55, 465–475.
138 Okada, H., Takemura, G., Kosai, K., Li, Y., Takahashi, T., Esaki, M., Yuge, K., Miyata, S., Maruyama, R., Mikami, A., Minaguchi, S., Fujiwar, T. and Fujiwara, H. (2005) Postinfarction gene therapy against transforming growth factor-β signal modulates infarct tissue dynamics and attenuates left ventricular remodeling and heart failure. Circulation 111, 2430–2437.
139 Liao, R. L. (2005) Yin and yang of myocardial transforming growth factor-β1: timing is everything. Circulation 111, 2416–2417.
140 Bunnell, B. A., Askari, F. K. and Wilson, J. M. (1992) Targeted delivery of antisense oligonucleotides by molecular conjugates. Somat. Cell Mol. Genet 18, 559–569.
141 Dean, N. M. and McKay, R. (1994) Inhibition of protein kinase C-α expression in mice after systemic administration of phosphorothioate antisense oligodeoxynuceotides. Proc. Natl. Acad. Sci. USA 91, 11762–11766.
142 Tzai, T. S., Lin, C. I., Shiau, A. L., Wu, C. L. (1998) Antisense oligonucleotide specific for transforming growth factor-beta 1 inhibit both *in vitro* and *in vivo* growth of MBT-2 murine bladder cancer. Anticancer Res. 18, 1585–1589.
143 Ihn, H., Yamane, K., Kubo, M. and Tamaki, K. (2001) Blockade of endogenous transforming growth factor-beta signaling prevents up-regulated collagen synthesis in scleroderma fibroblasts: association with increased expression of transforming growth factor-beta receptors. Arthritis Rheum. 44, 474–480.
144 Kurreck, J. (2003) Antisense technologies improvement through novel chemical modifications. Eur. J. Biochem. 270, 1628–1644.
145 Iyer, S. N, Margolin, S. B., Hyde, D. M. and Giri, S. D. (1998) Lung fibrosis is ameliorated by pirfenidone fed in diet after the second dose in a three-dose bleomycin-hamster model. Exp. Lund Res. 24, 119–132.
146 Azuma, A., Nukiwa, T., Tsuboi, E., Suga, M., Abe, S., Nakata, K., Taguchi, Y., Nagai, S., Itoh, H., Ohi, M., Sato, A. and Kudoh, S. (2005) Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis Am. J. Respir. Crit. Care Med. 171, 1040–1047.
147 Kakugawa, T., Mukae, H., Hayashi, T., Ishii, H., Abe, K., Fujii, T., Oku, H., Miyazaki, M., Kadota, J. and Kohno, S. (2004) Pirfenidone attenuates expression of HSP47 in murine bleomycin-induced pulmonary fibrosis. Eur. Respir. J. 24, 57–65.
148 Iyer, S. N., Gurujeyalakshmi, G. and Giri, S. N. (1999) Effects of pirfenidone on transforming growth factor-β gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. J. Pharmacol. Exp. Ther. 291, 367–373.