



TGF- β and fibrosis in different organs - molecular pathway imprints

Dirk Pohlers, Julia Brenmoehl, Ivonne Löffler, Cornelia K. Müller, Carola Leipner, Stefan Schultze-Mosgau, Andreas Stallmach, Raimund W. Kinne, Gunter Wolf

► To cite this version:

Dirk Pohlers, Julia Brenmoehl, Ivonne Löffler, Cornelia K. Müller, Carola Leipner, et al.. TGF- β and fibrosis in different organs - molecular pathway imprints. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2009, 1792 (8), pp.746. 10.1016/j.bbadis.2009.06.004 . hal-00506515

HAL Id: hal-00506515

<https://hal.science/hal-00506515>

Submitted on 28 Jul 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

TGF- β and fibrosis in different organs - molecular pathway imprints

Dirk Pohlers, Julia Brenmoehl, Ivonne Löffler, Cornelia K. Müller, Carola Leipner, Stefan Schultze-Mosgau, Andreas Stallmach, Raimund W. Kinne, Gunter Wolf

PII: S0925-4439(09)00135-5
DOI: doi:[10.1016/j.bbadis.2009.06.004](https://doi.org/10.1016/j.bbadis.2009.06.004)
Reference: BBADIS 62965

To appear in: *BBA - Molecular Basis of Disease*

Received date: 2 March 2009
Revised date: 11 June 2009
Accepted date: 12 June 2009



Please cite this article as: Dirk Pohlers, Julia Brenmoehl, Ivonne Löffler, Cornelia K. Müller, Carola Leipner, Stefan Schultze-Mosgau, Andreas Stallmach, Raimund W. Kinne, Gunter Wolf, TGF- β and fibrosis in different organs - molecular pathway imprints, *BBA - Molecular Basis of Disease* (2009), doi:[10.1016/j.bbadis.2009.06.004](https://doi.org/10.1016/j.bbadis.2009.06.004)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TGF- β and fibrosis in different organs – molecular pathway imprints

Dirk Pohlers¹, Julia Brenmoehl², Ivonne Löffler³, Cornelia K. Müller⁴, Carola Leipner⁵,
Stefan Schultze-Mosgau⁴, Andreas Stallmach², Raimund W. Kinne¹, Gunter Wolf³

¹ Experimental Rheumatology Group, Department of Orthopedics, Waldkrankenhaus “Rudolf Elle” Eisenberg, University Hospital, Friedrich Schiller University, Jena

² Department of Gastroenterology, Hepatology and Infectious Diseases, Clinic of Internal Medicine II, University Hospital, Friedrich Schiller University, Jena

³ Clinic of Internal Medicine III, University Hospital, Friedrich Schiller University, Jena

⁴ Clinic of Oromaxillofacial Surgery/Plastic Surgery, University Hospital, Friedrich Schiller University, Jena

⁵ Institute of Animal Research, University Hospital, Friedrich Schiller University, Jena

Corresponding author:

Raimund W. Kinne

Experimental Rheumatology Unit, University Hospital Jena

Department of Orthopedics, Waldkrankenhaus “Rudolf Elle”

Klosterlausnitzer Str. 81

07607 Eisenberg

e-mail: Raimund.W.Kinne@med.uni-jena.de

Tel./Fax +49 36691 81228/6

Key words: TGF- β , fibrosis, arthritis, nephritis, inflammatory bowel disease, Crohn’s disease, wound-healing, myocarditis

ABSTRACT

The action of transforming-growth-factor (TGF)- β following inflammatory responses is characterized by increased production of extracellular matrix (ECM) components, as well as mesenchymal cell proliferation, migration, and accumulation. Thus, TGF- β is important for the induction of fibrosis often associated with chronic phases of inflammatory diseases. This common feature of TGF-related pathologies is observed in many different organs. Therefore, in addition to the description of the common TGF- β -pathway, this review focuses on TGF- β -related pathogenetic effects in different pathologies/organs, i. e., arthritis, diabetic nephropathy, colitis/Crohn's disease, radiation-induced fibrosis, and myocarditis (including their similarities and dissimilarities). However, TGF- β exhibits both exacerbating and ameliorating features, depending on the phase of disease and the site of action. Due to its central role in severe fibrotic diseases, TGF- β nevertheless remains an attractive therapeutic target, if targeted locally and during the fibrotic phase of disease.

1. INTRODUCTION

The fibrotic reaction of the connective tissue following an inflammatory response is mainly characterized by an increased production of extracellular matrix (ECM) components and mesenchymal cell proliferation, migration and accumulation. Despite the existence of numerous distinct causes of chronic inflammatory diseases in different organs and tissues, these diseases are generally characterized by: i) severe and intermittent progression with phases of acute exacerbation and remission; ii) immigration of inflammatory cells (macrophages, granulocytes and T-cells); and iii) increased expression of pro-inflammatory mediators (Figure 1). These processes result in the proliferation of local fibroblasts and, by interaction with epithelial cells, in their differentiation into myofibroblasts and can be regarded as a misguided wound healing. Finally, the inflammatory process comes to rest, but the massive fibrosis prevents a rebuilding of functionally intact tissue and organs.

Transforming growth factor (TGF)- β is an ubiquitously expressed cytokine belonging to a large superfamily of activins/bone morphogenetic proteins [1]. This mediator plays an active role in the processes discussed above, such as proliferation, wound healing [2], and synthesis of ECM molecules [3]. TGF- β , therefore, strongly contributes to fibrotic disorders such as diabetic nephropathy, Crohn's disease, rheumatoid arthritis, radiation-induced fibrosis, and myocarditis. However, TGF- β is clearly a bi- (or multi-) functional molecule with strong effects on the immune system [4,5].

2. TGF- β SIGNALING PATHWAY

TGF- β is synthesized as one part of a large molecule, the pro-TGF- β containing the latency-associated proteins (LAP; Figure 2). The latter is cleaved from TGF- β in the Golgi apparatus, but remains non-covalently associated with the growth factor. The disulfide-bound latent-

TGF- β -binding proteins 1/2 (LTBP 1/2) connect the whole complex to the ECM (details described in [6]). Release of TGF- β from the pro-TGF- β complex can be achieved through proteolytic activity by plasmin [7] or matrix-metalloproteinase (MMP)-2 and MMP-9 [8], through integrins [9,10], recently reviewed in [11], treatment with mild acids [12], or through the action of thrombospondin (THBS; [13]) by disrupting the non-covalent interactions between LAP and TGF- β 1. Once released, TGF- β mediates signals through pairs of type I and type II receptors [14]. The type III receptor (betaglycan) acts - probably in conjunction with other heparan sulfate glycans like syndecan [15]- as a co-receptor for binding/presenting TGF and a regulator of TGF- β signaling [16]. The result of ligand binding is the activation of the type II receptor (TGFBR2), which then phosphorylates the type I receptor (TGFBR1). The active receptor complex then phosphorylates the so-called R (receptor)-Smad-2 or Smad-3 that propagates the signal [17]. The phosphorylation of Smad2/3 decreases the affinity for the Smad-anchor for receptor activation (SARA), which in non-stimulated cells mediates the retention of Smad2/3 in the cytoplasm by interaction, and increases the affinity of Smad2/3 for Smad-4 (a so-called co-Smad). This complex is now able to enter the nucleus, to bind transcriptional co-activators like p300 and Creb-binding-protein (CBP) or repressors like SkiL or TGIF [18], and to regulate the transcriptional activity of various genes.

Mitogen-activated protein kinases (MAPKs) and protein kinase C can also interfere with either the nuclear translocation or binding of Smad3/4 complexes to DNA and regulate TGF- β 1 signalling [19,20]. Moreover, the serine-threonine protein kinase B can directly interact with Smad3, thereby preventing its phosphorylation and nuclear translocation [21]. Other pathways can be directly activated by TGF- β 1. These include components of the MAPK pathway, such as ras, raf, ERK, p38 and JNK, the phosphatidylinositol-3 kinase cascade, as well as the regulators of cadherin junctions, RhoA and Rac ([19,22,23]; Fig. 1). Recently, an involvement of the focal adhesion kinase (FAK) was established in

myofibroblast differentiation and in remodeling of the connective tissue following stimulation with TGF [24,25]. In line with these results, TGF-induced FAK-signaling is required for the activation of TAK [26] or MEKK1 and, subsequently, of JNK [27], all factors shown to be essential for the transcription of pro-fibrotic genes.

Notably, that signal transduction pathways have their own intracellular regulators. An inhibitory Smad (Smad7) blocks TGF- β 1 signaling by physical interaction with the activated TGFBR1 receptor and prevents the docking and phosphorylation of Smad2/3 [28,29].

This complex TGF- β signaling pathway (Figure 2) contains numerous ligands, receptors, and signaling molecules which, as potential targets of dysregulation via increased or decreased expression, activation or interaction, may be partially involved in fibrotic reactions in the diseases discussed below.

3. TGF- β -RELATED MOLECULES IN RHEUMATOID ARTHRITIS

The importance of TGF- β 1 in rheumatoid arthritis (RA) ranges from an association with certain vascularization patterns in the synovial membrane (SM) [30], and an association of TGF- β polymorphisms with the radiological signs of joint destruction [31] to an induction of pro-inflammatory cytokines, MMP [32], aggrecanase [33] and urokinase-type plasminogen activator [34]. In addition, TGF- β plays an important role for the function of regulatory T-cells [5] in the suppression of autoimmunity. Indeed, increased levels of TGF- β 1 have been found in the synovial membrane of patients with RA by northern blot [35], immunohistochemistry [36,37], western blot [38] and in synovial effusions [39]. Also TGFBR2 was detected at higher levels than in normal synovial tissue [35].

Using “pathway-directed” software following genome-wide comparison between synovial fibroblasts (SFB) from patients with RA and osteoarthritis (OA) with Affymetrix

arrays, gene expression of TGF- β 1, TGF- β 3, LTBP1/2, THBS1, TGFBR1, SARA, CBP, SkiL – belonging to the TGF- β -pathway (Figure 2) - was elevated in RA [40]. After validating array data at the mRNA and protein levels using quantitative PCR and western blot/immunohistochemistry, we confirmed an upregulated TGF- β pathway in RA-SFB. The presence of TGF- β 1, in conjunction with increased amounts of TGF- β -releasing THBS1 and a higher expression of TGFBR1, may thus lead to an amplified response of RA-SFB to TGF- β . This RA-specific response has been confirmed by increased expression of MMP-11 following TGF- β stimulation, implying a pathogenetic relevance of the TGF- β -pathway for MMP-induced degradation or remodeling processes in RA [40].

The importance of TGF- β for the pathogenesis of arthritis is emphasized by a number of animal models. The abundant expression of TGF- β 1, 2, and 3 as well as the TGFBR1 and -2 in rat synovium was increased after onset of the collagen-induced arthritis (CIA) [41], and direct intra-articular injection of TGF- β 1 or TGF- β 2 induced synovial erythema, swelling, and cellular infiltration resulting in synovial inflammation and hyperplasia [42]. Conversely, neutralization of TGF- β inhibited acute and chronic arthritis induced by streptococcal cell wall (SCW) [43]. In line with these observations, an adenovirus-mediated overexpression of TGF- β 1 in rabbit knees led to an increased glycosaminoglycan release, nitric oxide production and, most notably, to fibrosis and muscle edema [44]. The prevention of CIA by administration of a TGFBR1 inhibitor (HTS466284), which concomitantly reduced the expression of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-AA, TNF- α , and cellular proliferation [45], further underlines the pathogenetic role of TGF- β in arthritis.

In contrast to the above-mentioned studies, numerous observations show beneficial effects of TGF- β in arthritis. If administered systemically, TGF- β 1 suppressed SCW-induced

arthritis, as measured by cellular infiltration and joint erosion [46]. In addition, investigation of the cytokine expression during CIA demonstrated a strong upregulation of TGF- β 1/2 in the remission state of disease, possibly reflecting the anti-inflammatory regulation of T-cells by TGF- β in arthritis. If TGF- β signaling was inhibited by expression of dominant-negative TGFBR2 in T-cells [47] the susceptibility and the clinical severity of CIA was strongly increased. Likewise, if TGF- β 1 was retrovirally overexpressed in arthritogenic splenocytes, CIA could not be transferred to SCID mice and established disease was ameliorated [48]. Therefore, it may be important to inhibit TGF- β only at the site of inflammation without targeting regulatory lymphocytes at extra-articular sites.

4. TGF- β -RELATED MOLECULES IN DIABETIC NEPHROPATHY

TGF- β and its signal transduction play a major role in diabetic nephropathy and have therefore been thoroughly studied [49-53]. Among the features of the diabetic milieu, hyperglycemia, increased non-enzymatic glycation of proteins, *de novo* synthesis of diacylglycerol and subsequent activation of protein kinase C, increased intracellular glucosamine production, and enhanced renal production of vasoactive agents (angiotensin II, endothelins, thromboxane) have all been shown to increase the expression of TGF- β in cultured renal cells and animal models of diabetic nephropathy [50,52,54].

The TGF- β level is elevated in the kidneys of insulin-dependent diabetic animals during both early and late stages of disease [53,54]. Treatment of the streptozotocin (STZ)-diabetic rat with sufficient insulin to reduce hyperglycemia suppressed the enhanced expression of TGF- β and matrix components in the glomeruli. In the STZ-diabetic rat and mouse, increased TGF- β 1 expression in the renal cortex and glomeruli as well as up-regulation of the TGFBR2 mRNA and protein was noted early after the onset of diabetes

[53].. The *db/db* mouse, a model of type 2 diabetes, characterized by hyperglycemia, obesity, and insulin resistance, develops increased amounts of TGF- β 1 localized in the glomerular compartments [55]. In contrast, the mRNA and protein levels of the TGFBR2 are significantly up-regulated in both the glomerular and the tubulointerstitial compartments [55].

The development of diabetic renal hypertrophy and glomerulosclerosis is likely caused by heightened activity of the TGF- β system [56-63]. Short-term treatment of the STZ-diabetic mouse with a neutralizing monoclonal antibody against all three isoforms of TGF- β prevented glomerular hypertrophy, reduced the increment in kidney weight by 50%, and significantly attenuated the increase in TGF- β 1, α 1(IV) collagen, and fibronectin mRNAs without affecting glycemic control [56]. The results of this study suggested a cause-and-effect relationship between the renal TGF- β system and the development of early structural changes in diabetic nephropathy. Systemic anti-TGF- β therapy for eight weeks prevented the mesangial matrix expansion of diabetic glomerulosclerosis and, most importantly, preserved kidney function, showing for the first time that neutralization of TGF- β activity prevents the progression of renal failure in diabetes [57]. However, the anti-TGF- β antibody did not reduce albuminuria, which itself may promote the progression of renal insufficiency [57]. The paradox of preserved renal function in view of persistent albuminuria may be explained by postulating that the deleterious effects of proteinuria are themselves mediated by the TGF- β system [63-65].

In mouse mesangial and tubular cells, high glucose stimulates the transcription of fibronectin and, in addition, potentiates the transcriptional activation of fibronectin by TGF- β 1 [58,59]. This particular effect of TGF- β 1 appears to be mediated by Smad3, because over-expression of Smad3 alone was able to induce fibronectin promoter activity [66]. In conjunction with exogenous TGF- β 1, Smad3 over-expression synergistically increased

fibronectin expression, as if the extra Smad3 had increased the efficiency of TGF- β signaling [66]. Finally, transfection of a Smad3-dominant-negative construct inhibited TGF- β 1 stimulated fibronectin promoter activity [67,68]. However, part of the TGF- β 1-induced fibronectin expression may be mediated in parallel by the p38MAPK pathway [68,69]. Finally, there is evidence that Smad3 is a central mediator in the TGF- β 1-induced increase of mRNA expression for α 1(I) collagen [68,70]. TGF- β -induced MAPK activation also leads to N-terminal phosphorylation of p53 that enables its interaction with TGF- β -activated Smads [64], an indication for cross-talk between the various TGF- β signaling pathways.

TGF- β 1 may have additional effects besides the stimulation of extracellular matrix production. In podocytes, TGF- β 1 induces apoptosis through Smad7 by inhibiting nuclear translocation of the cell survival factor NF- κ B [70]. TGF- β 1-mediated activation of Smad7 is specific for podocytes and is not found in mesangial cells in a limited series of biopsies from patients with diabetic nephropathy [64]. Since podocytes are terminally differentiated cells unable to undergo cell division due to the up-regulation of the cell cycle inhibitory proteins p57 and p27^{Kip1}, apoptotic loss of cells was not replaced.

Studies performed in diabetic patients with various degrees of nephropathy also underline the importance of the renal TGF- β system in disease development [71-73]. All three isoforms of TGF- β are elevated in both the glomerular and the tubulointerstitial compartments of patients with established diabetic nephropathy [71,72]. Furthermore, glomerular TGF- β 1 mRNA is markedly increased in biopsy specimens from patients with proven diabetic kidney disease. These investigations suggests that increased renal TGF- β levels closely correlate with the degree of mesangial matrix expansion, interstitial fibrosis, and renal insufficiency.

Another study, designed to assess renal production of TGF- β [73] measured aortic, renal vein, and urinary levels of TGF- β in 14 type 2 diabetic and 11 non-diabetic control patients undergoing elective coronary artery catheterization [73]. Both groups were roughly matched with regard to the range of renal function and the presence of hypertension and proteinuria. Renal blood flow was measured to calculate the net mass balance across the kidney. The gradient of TGF- β 1 concentration across the renal vascular bed was negative in the non-diabetic patients indicating net renal extraction of TGF- β 1, whereas the gradient was positive in the diabetic patients indicating net renal production of TGF- β 1. When the renal TGF- β 1 mass balance was calculated, a similar pattern was observed with the non-diabetic kidney removing approximately 3500 ng/min of TGF- β 1 from the circulation, and the diabetic kidney adding approximately 1000 ng/min of TGF- β 1 [73]. In addition, the level of bioassayable TGF- β was increased four-fold in the urine of diabetic *versus* non-diabetic patients. This was not simply a function of enhanced glomerular permeability to protein since diabetic patients both with and without microalbuminuria displayed similarly high rates of urinary TGF- β excretion [73]. These results demonstrated that the kidneys of diabetic patients overproduce TGF- β 1 protein; further details, e. g., the exact contribution of the different renal cell types, need to be investigated.

An interesting *post-hoc* study assessed whether treatment with the angiotensin converting enzyme inhibitor captopril would lower serum TGF- β 1 levels in a small subset of patients with diabetic nephropathy who had been enrolled in the Collaborative Study Group [74,75]. After six months, the serum TGF- β 1 level decreased significantly by 21% in the captopril-treated group, whereas it increased slightly by 11% in the placebo-treated group [75]. Interestingly, the captopril-treated patients with decreased serum TGF- β 1 levels tended to have better preserved renal function over the ensuing two-year period [75]. This association

was even more pronounced in the subset of patients with an initial glomerular filtration rate of less than 75 ml/min. These results suggest that TGF- β 1 plays a pivotal role in the progression of diabetic nephropathy and that angiotensin converting enzyme inhibitor therapy may protect the kidney by lowering TGF- β 1 production.

5. TGF- β -RELATED MOLECULES IN INTESTINAL INFLAMMATION – A DOUBLE-SIDED SWORD

TGF- β is constitutively expressed by epithelial cells, fibroblasts, and mononuclear cells in the gastrointestinal tract [76]. Its critical role in intestinal homeostasis as a negative master regulator of inflammation is well-established [77,78] and indisputable. However, translation of elegant mouse experiments into therapeutic interventions in humans requires a clearer understanding of TGF- β activity in the human gut. Mice with global TGF- β defects, such as TGF- β -null mice or transgenic mice expressing a dominant negative TGFBR2 chain, are unresponsive to TGF- β 1 signaling. The former die soon after birth due to systemic inflammation, and the latter develop severe colonic and pulmonary inflammation [79,80]. These manifestations of global TGF- β 1 defects are mirrored in mouse models of colitis in that the secretion of TGF- β 1 is consistently associated with either the protection from colitis or a greatly diminished severity of colitis. This is seen both in the T_H1 model of colitis induced by the haptening reagent trinitrobenzene sulfonic acid (TNBS), which mimics Crohn's disease (CD), or the T_H2 model of colitis induced by the haptening agent oxazolone, which mimics ulcerative colitis (UC) [81,82]. In addition, it is seen in the colitis of SCID or RAG2-deficient mice receiving CD45RB^{high} (naive) T cells in which the protective effect of the cotransfer of CD45RB^{low} T (memory) cells is abolished by concomitant administration of a neutralizing TGF- β 1 antibody [83]. These and other studies quite conclusively establish that TGF- β 1 plays

an essential, regulatory role in the control of colitis. On the other hand, in mice TGF- β 1 in combination with IL-6 induces a strong pro-inflammatory T_H17 cell differentiation [84-86]. Notably, in humans TGF- β 1 does not have a direct effect on the development of human T_H17 cells, but it can indirectly favour the development of these cells by suppressing the expression of T-bet and selectively inhibiting the expansion of IFN- γ -producing T cells [87-89].

The important role of Smad3 as an essential mediator of TGF- β 1-induced anti-inflammatory and suppressive activities at the mucosal level emerges from studies in mice with targeted deletion of the Smad3 gene. The animals are viable, but die from defects in mucosal immunity at 1–6 months of age. Mutant mice show diminished cell responsiveness to TGF- β 1, massive infiltration of T cells, and multiple pyogenic abscesses in the stomach and intestine [87,90]. When Smad signaling was studied in normal human gut mucosa, whole biopsies or isolated lamina propria mononuclear cells, a basal level of phosphorylated (phospho-) Smad3 was observed which was rapidly upregulated by the addition of exogenous TGF- β 1 [91].

TGF- β has been also implicated as a key inducer of epithelial-mesenchymal transition (EMT) [92-97]. Amongst others, EMT is an essential component of tissue remodeling and wound repair (reviewed in [98,99]) and fibrosis (reviewed in [100]). During this transition, the epithelial phenotype, characterized by strong cell-cell junctions and polarity, is replaced by a mesenchymal phenotype, with reduced cell-cell interactions, a fibroblastic morphology and increased motility. TGF- β stimulates the proliferation of many cell types, particularly those of mesenchymal origin, and it is also a potent inhibitor of epithelial cell proliferation. EMT in response to TGF- β 1 and in fibrosis is mediated predominantly via Smad-dependent (mainly Smad3) pathways [101,102]. A loss of Smad3 in mice blocked both morphological

changes of lens epithelium to a mesenchymal phenotype and expression of EMT markers in response to injury *in vivo* or to exposure to exogenous TGF- β in organ culture [101].

CD is a chronic, progressive disease of the gastrointestinal tract with an unknown etiology. It is characterized by transmural inflammation of all layers of the bowel wall. The formation of stenoses and strictures is common in this disease, which causes abdominal pain, anorexia, and weight loss. Approximately 50% of CD patients undergo surgery for this type of complication during a 10-year course; however, the recurrence rate after surgery is high. In contrast, UC rarely causes intestinal stenosis. Cytokines released from inflammatory cells have long been implicated in the pathogenesis of intestinal fibrosis. TGF- β /Smad signaling plays an important role in CD [103-105]. The transmural infiltrate of CD is responsible for initiating and maintaining a series of connective tissue changes not only involving the mucosa, but also the submucosa and *muscularis mucosae* and *muscularis propria*, where a marked increase of collagen type I, III, and V mRNA is observed [106,107]. TGF- β has been identified as one of the central growth factors/cytokines that specifically induces a fibrotic response after inflammatory injury in the intestinal tract. In CD, there is a marked overexpression of TGF- β 1 and TGFBRs in the colonic mucosa [76,108]. Fibrosis in CD can therefore be interpreted as an aberrant healing response to mucosal injury [109]. In addition, TGF- β appears to be involved in intestinal fibrosis in other enteropathies, such as radiation enteritis, collagenous colitis, and intestinal graft-versus host disease [110-112]. Both TGF- β and its receptors are overexpressed in the intestine of patients with CD [113]. Intestinal fibroblast expression of TGF- β isoforms varies according to the nature of tissue. Fibroblasts from normal and inflamed mucosa both express the TGF- β 1 and TGF- β 3 isoforms, while those from fibrotic tissue show reduced expression of TGF- β 3, but enhanced expression of TGF- β 2 and TGF- β 1 [114]. This is remarkable, since the TGF- β 1 and TGF- β 2 isoforms have

been specifically implicated in pathogenic fibrosis, while TGF- β 3 appears to have antifibrotic properties [115]. Monteleone and colleagues have provided insight into the failure of TGF- β down-regulation in CD. Despite the abundant expression of TGF- β in the mucosa of patients with CD, phospho-Smad3 is diminished in the mucosa compared to control mucosal samples, as is the complex of Smad3 with Smad4. This may be due to the induction and overexpression of Smad7 in the mucosa of patients with CD and UCs [116]. However, upregulation of Smad7 is not specific for inflammatory bowel disease (IBD), but also occurs in *Helicobacter pylori*-induced gastritis [117]. In addition, mucosal T cells in both whole tissue and isolated cells show defective TGF- β 1 signaling as measured by reduced immuno-reactivity against phospho-Smad3 [117]. Specific anti-sense oligonucleotides for Smad7 reduce expression of Smad7 in cells isolated from IBD patients which then become responsive to exogenous TGF- β 1. TGF- β 1 cannot inhibit pro-inflammatory cytokine production in isolated lamina propria mononuclear cells from CD patients, but inhibition of Smad7 with anti-sense oligonucleotides restores TGF- β 1 signaling and allows TGF- β 1 to inhibit cytokine production. In inflamed mucosal tissue explants from CD patients, inhibition of Smad7 also restores phospho-Smad3 and decreases pro-inflammatory cytokine production, an effect which is partially blocked by anti-TGF- β 1. The extension of these studies examined the interactions between Smad signaling and NF- κ B activation in inflamed gut: While TGF- β 1 is a potent inhibitor of TNF- α -induced NF- κ B activation in normal gut, it has no activity in inflamed gut. This can be attributed to over-expression of Smad7, since treatment of cells from inflamed gut with anti-sense to Smad7 allows TGF- β 1 to rapidly down-regulate NF- κ B activation [117].

If it becomes possible to specifically inhibit Smad7, endogenous TGF- β 1 in the inflamed gut may negatively regulate pro-inflammatory cytokine production and NF- κ B activation, the major components of the immune overactivity which drives tissue injury in

IBD. At the same time, however, it is important to discover the factors which control Smad7 expression in the inflamed gut. Furthermore, cell-specific expression of Smad7 will be important because TGF- β 1 has different effects on different cell types. Thus, while blocking Smad7 will allow TGF- β 1 to reduce pro-inflammatory cytokine production by T cells and macrophages, it may allow TGF- β 1 to increase collagen production in myofibroblasts, resulting in fibrosis. However, at the moment the relative importance of the known inducers of Smad7 in the gut, such as IFN- γ or TNF- α , or even whether TGF- β 1 itself induces Smad7 in a negative regulatory loop, is still unclear.

6. TGF- β -RELATED MOLECULES IN RADIATION-INDUCED FIBROSIS

TGF- β 1 levels are increased in irradiated mouse skin [118,119] and decrease slowly after irradiation in both pig and human skin [120,121]. Following microvascular hard or soft tissue transfer, TGF- β 1 is again upregulated in a biphasic manner. The first expression peak on day 3 post operation is due to enhanced activation of latent TGF- β 1 by extracellular enzymes while the second between day 14 and 28 after surgery is a result of *de novo* synthesis [122]. Its most important signaling receptor TGFBR2 is upregulated in irradiated graft beds as well [123]. Signaling leads to increased nucleoplasmatic shuttling of active Smad2/3 and induction of TGF- β 1 target genes in fibrotic healing, which is mainly due to decrease in cytoplasmatic levels of the inhibitory Smad7 [124].

As a consequence, the extracellular matrix is qualitatively and quantitatively altered [125]. Prolyl-hydroxyprolinase- β overexpression [126] promotes synthesis of collagen I, III and IV [124], while repression of degrading enzymes, such as MMP-1 and induction of tissue inhibitors [127] suppresses the degrading pathways. Moreover, integrin surface receptors, such as α 2 β 1 integrin are up-regulated as well and modulate transmission of tensile forces

[128]. In the presence of such forces fibroblasts differentiate into myofibroblasts resulting in constrictive fibrosis [129].

Irradiation-induced fibrosis and damage to the microvasculature lead to wound healing disorders following surgery in previously irradiated areas. Such disorders are dependent, in part, on the radiation dosage and the timing of surgery after irradiation [130,131] and reduce the success rate of free flaps to 90% compared with 94% in non-irradiated graft beds [132].

Taking the central role of TGF- β 1 in radiation-impaired wound healing into account, the question of whether TGF- β 1-levels differ in the healthy tissue between different patients is of utmost importance. It has been demonstrated that patients with an increased TGF- β 1 plasma level exhibit an increased risk of developing skin fibrosis following irradiation [133]. To find a particular predictor which sufficiently corresponds with the frequency to develop wound healing complications following surgery in previously irradiated graft beds would give a large clinical impact on planning individual treatment protocols. The availability of reliable markers may eventually allow the prediction of outcome prior to commencement of treatment, and thus allow modification of combined protocols to minimize late adverse effects without compromising tumor control.

7. TGF- β -RELATED MOLECULES IN MYOCARDITIS

Heart fibrosis is a hallmark feature of the chronic stage of viral myocarditis [134,135]. Human pathogenic coxsackievirus B3 (CVB3) is considered the most frequent viral cause of chronic myocarditis in men [136]. Clinical manifestations of acute myocarditis vary from flu-like symptoms to the fulminant fatal forms. Frequently, acute myocarditis, with distinct onset, follows a monophasic clinical course, and the majority of patients recover spontaneously after several days of congestive heart failure. Some patients progress into subacute or chronic

forms, which ultimately lead to death. The molecular mechanisms underlying fibrosis development in chronic myocarditis are currently not well understood.

Like humans, mice develop a marked age-related susceptibility to CVB infections [137]. The myocardial lesions in mice closely resemble those seen in human disease [138]; therefore, experimental murine models of coxsackievirus-induced myocarditis have been developed to investigate the pathogenesis of this disease. Although chronic inflammation is characteristic for the human disease as well as for our mouse model, fibrosis is more prevalent in the latter.

Excessive fibrosis, as it occurs under conditions of chronic myocarditis, can be classified as either replacement fibrosis, when functional tissue is replaced by connective tissue, or as reactive fibrosis, which is part of an adaptive process [139]. In addition to collagen, tenascin C, and fibronectin, splice variants of these molecules are often part of the fibrotic tissue [140,141]. Various cytokines and growth factors are believed to contribute to the induction of fibrosis, including TGF- β [142], IL-1, [143], TNF- α [144,145], and PDGFs [146,147]. For example, persistent expression of cytokines in the chronic stage of CVB3-induced myocarditis has been described for various mouse models [148-150], as well as for human dilatative cardiomyopathy [151]. New observations suggest that sustained pro-inflammatory signaling is associated with a pro-fibrotic phenotype based on TGF- β -mediated signaling [152].

For fibrogenesis in the heart, members of the PDGF family are apparently important mediators. Transgenic overexpression of PDGF-C and PDGF-D, two more recently discovered PDGF isoforms [153], in the heart leads to massive cardiac fibrosis [146,147]. Therefore, the importance of PDGF for the pathology of chronic myocarditis was investigated in mice with CVB3-induced myocarditis. Interestingly, all analysed isoforms of PDGF-A, -B, and -C were upregulated in close correlation with the inflammatory process. High levels of

the growth factors persisted only in MHC class II knockout mice, which develop a chronic myocarditis upon CVB3 infection, whereas immunocompetent C57BL/6 wild-type mice exhibit only an acute, completely reversible myocarditis [154,155].

Furthermore, it has been shown that the PDGF-receptor (PDGFR) blocker Imatinib inhibits activation of resident PDGFR, and attenuates fibrosis in this mouse model significantly [156]. These data strongly suggest that elevation of PDGF levels and subsequent activation of PDGFR causally contribute to the type of cardiac fibrosis which occurs in this model. Efficacy of Imatinib for attenuation of fibrosis has recently been reported also for other organs, i.e., liver, joints, and kidney [157-159]. It can therefore be assumed that Imatinib-sensitive tyrosine kinases play a more general role in fibrogenesis. In addition to the PDGFR [157,160], the Abelson tyrosine kinase, a mediator of TGF- β -signaling (c-Abl) [161], has also been proposed as a relevant target for Imatinib in the inhibition of fibrogenesis [159,162]. While our data [156], and data of others [146,147] strongly suggest a causal involvement of the PDGFR in fibrogenesis, they do not exclude that c-Abl activity is also involved in fibrogenetic signalling. It could function downstream of TGFBRs, but also partially mediate signalling of the activated PDGFR [163]. It is known that TGF- β can drive cardiac fibrosis when overexpressed in the mouse heart [164] and the use of genetic mouse models to understand the role of TGF- β signalling in the heart is reviewed in [165]. But the relative contribution of the different TGF- β -mediated signalling mechanisms to fibrosis in our model of CVB3-induced chronic myocarditis remains to be fully elucidated.

8. DIFFERENCES AND SIMILARITIES OF TGF- β IN DIFFERENT ORGANS

As presented in Table 1, numerous molecules have similar expression patterns in the diseases mentioned in this review. Due to limited information from the human system, some data were replaced by results derived from the respective animal model (fibrosis following irradiation).

TGF- β 1 is elevated in most pathologies in its active or latent form and shows similarities with the isoform TGF- β 2 in most cases. In contrast, the expression of TGF- β 3 varies from down-regulation during wound healing and CD, abundance without regulation in rheumatoid arthritis and UC, to up-regulation during diabetic nephropathy. If determined, the expression of TGFBR2 is often enhanced during disease. The widespread appearance of the term “n.d.” points out the urgent need for further investigation of the expression and the effects of TGF- β -related molecules in human diseases associated with fibrosis, particularly at the stage of signaling and transcriptional regulation.

9. THERAPEUTIC STRATEGIES

As either increased or decreased activity of the TGF- β pathway has been implicated in the pathogenesis of different human diseases, methods for increasing or decreasing signaling through these pathways are required [166]. There are some tools for enhancing TGF- β signals, e. g., direct administration of the ligand, usage of agonists, and increased expression of receptors or decreased expression of signaling antagonists.

On the other hand, there are different ways to inhibit TGF- β signaling, e. g., administration of neutralizing antibodies, application of soluble receptors, usage of antisense nucleotides, and chemically synthesized inhibitors of the receptor serine/threonine kinases.

Regarding TGF- β inhibition, some clinical trials have been performed with neutralizing antibodies, especially in fibrotic diseases, including a TGF- β 2 neutralizing antibody (lirdelimumab) which effectively decreased the amount of scarring after glaucoma surgery [167]. A TGF- β 1 neutralizing antibody (CAT-192, metelimumab) has been administered intravenously to patients with systemic sclerosis, which causes scarring in skin and internal organs, however, without any evidence of efficacy and with more adverse events

and serious adverse events in *verum* than in placebo patients [168]. This raises the question of whether broader or even complete blockade of the TGF- β axis will be safe or rather associated with substantial toxicity [168]. Despite these concerns, a pan-TGF- β (GC-1008) neutralizing antibody has recently been administered to patients with idiopathic pulmonary fibrosis in a phase I clinical trial for safety evaluation [169] and is being administered to patients with focal segmental glomerulosclerosis [170], renal cell carcinoma, and malignant melanoma [171]; however, safety and efficacy data from these studies are not yet available. Similar to neutralizing antibodies, soluble TGF- β superfamily receptors abrogate signaling at the ligand level by binding the ligand and preventing it from binding to its cell surface receptors. Due to the lack of studies in humans, only data from animal models are available. For example, soluble TGFBR3 has demonstrated efficacy against renal damage progression associated with diabetes in mice [172]. Similarly, soluble TGFBR2 has anti-cancer effects in mice, as it suppresses the growth and metastasis of pancreatic cancer cells [173] and also inhibits breast cancer cell growth, migration, invasion, and metastasis [174]. Using anti-sense oligonucleotides to reduce the expression of TGF- β superfamily members is a relatively new therapeutic tool that has been already successfully applied in clinical trials for cancer treatment [175,176]. Also, small molecule inhibitors have been introduced to block TGF- β signaling. The TGFBR1-inhibitor Ly573636, which blocks intrinsic receptor-kinase activity, is currently being assessed in patients with certain cancers (see [176]), whereas other TGFBR1-inhibitors have shown to be efficient in mouse tumor models [177].

10. CONCLUSIONS

Due to the central role of TGF- β in severe fibrotic diseases (with similarities and dissimilarities among organs/diseases) it is an attractive therapeutic target, as already shown in several clinical trials (see above).

The strongly bifunctional role of TGF- β , however (pro-fibrotic, but anti-inflammatory), requires great care for the application of TGF- β -directed treatments. Future strategies will therefore have to focus on developing suitable tools to address this bifunctionality such as:

- 1) locally or regionally restricted administration of agonists or antagonists (in particular, broad TGF- β blockade by agents such as TGF- β antibodies, TGF- β receptors (soluble/anti-sense), latency-associated peptide or Smad7; 2) phase-dependent application in those periods of disease dominated by the critical pathogenetic features of TGF- β ; and 3) development of local or systemic biomarkers indicative of a future favorable response. The systemic applicability of broad TGF- β blockade will have to await the safety data from ongoing trials.

ACKNOWLEDGEMENTS

The authors thank Ulrike Körner, Simone Schönfelder, Soo-Yeon Lee, Christin Thiele and Nora Moll for excellent technical assistance. Monique Nürnberger and E. Palombo-Kinne are gratefully acknowledged for critical reading and /or editing of the manuscript. The work of R. W. Kinne was supported by the Deutsche Forschungsgemeinschaft (Ki439/6, Ki439/7), the Interdisciplinary Center of Clinical Research Jena (FKZ 010405), and the Jena Center of Bioinformatics (FKZ0313652B). G. Wolf received grants from the Deutsche Forschungsgemeinschaft (Wo 460/14-1, 14-2) and the Interdisciplinary Center of Clinical Research Jena.

REFERENCES

- [1] Y. Shi, J. Massague, Mechanisms of TGF-beta signaling from cell membrane to the nucleus, *Cell* 113 (2003) 685-700.
- [2] B.J. Falter, R.A. Macsata, D. Plummer, L. Mishra, A.N. Sidawy, Transforming growth factor-beta and wound healing, *Perspect. Vasc. Surg. Endovasc. Ther.* 18 (2006) 55-62.
- [3] B.A. Heccevar, P.H. Howe, Analysis of TGF-beta-mediated synthesis of extracellular matrix components, *Methods Mol. Biol.* 142 (2000) 55-65.
- [4] M.O. Li, R.A. Flavell, TGF-beta: a master of all T cell trades, *Cell* 134 (2008) 392-404.
- [5] S.M. Wahl, W. Chen, Transforming growth factor-beta-induced regulatory T cells referee inflammatory and autoimmune diseases, *Arthritis Res. Ther.* 7 (2005) 62-68.
- [6] J.P. Annes, J.S. Munger, D.B. Rifkin, Making sense of latent TGFbeta activation, *J. Cell Sci.* 116 (2003) 217-224.
- [7] Y. Sato, D.B. Rifkin, Inhibition of endothelial cell movement by pericytes and smooth muscle cells: activation of a latent transforming growth factor-beta 1-like molecule by plasmin during co-culture, *J. Cell Biol.* 109 (1989) 309-315.
- [8] Q. Yu, I. Stamenkovic, Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis, *Genes Dev.* 14 (2000) 163-176.
- [9] J.S. Munger, X. Huang, H. Kawakatsu, M.J. Griffiths, S.L. Dalton, J. Wu, J.F. Pittet, N. Kaminski, C. Garat, M.A. Matthay, D.B. Rifkin, D. Sheppard, The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis, *Cell* 96 (1999) 319-328.
- [10] D. Mu, S. Cambier, L. Fjellbirkeland, J.L. Baron, J.S. Munger, H. Kawakatsu, D. Sheppard, V.C. Broaddus, S.L. Nishimura, The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1, *J. Cell Biol.* 157 (2002) 493-507.
- [11] P.J. Wipff, B. Hinz, Integrins and the activation of latent transforming growth factor beta1 - an intimate relationship, *Eur. J. Cell Biol.* 87 (2008) 601-615.

- 525 [12] R.M. Lyons, J. Keski-Oja, H.L. Moses, Proteolytic activation of latent transforming
526 growth factor-beta from fibroblast-conditioned medium, *J. Cell Biol.* 106 (1988) 1659-
527 1665.
- 528 [13] J.E. Murphy-Ullrich, M. Poczatek, Activation of latent TGF-beta by thrombospondin-
529 1: mechanisms and physiology, *Cytokine Growth Factor Rev.* 11 (2000) 59-69.
- 530 [14] R. Derynck, Y.E. Zhang, Smad-dependent and Smad-independent pathways in TGF-
531 beta family signalling, *Nature* 425 (2003) 577-584.
- 532 [15] L. Chen, C. Klass, A. Woods, Syndecan-2 regulates transforming growth factor-beta
533 signaling, *J. Biol. Chem.* 279 (2004) 15715-15718.
- 534 [16] H.J. You, M.W. Bruinsma, T. How, J.H. Ostrander, G.C. Blobe, The type III TGF-
535 beta receptor signals through both Smad3 and the p38 MAP kinase pathways to
536 contribute to inhibition of cell proliferation, *Carcinogenesis* 28 (2007) 2491-2500.
- 537 [17] J. Massague, How cells read TGF-beta signals, *Nat. Rev. Mol. Cell Biol.* 1 (2000)
538 169-178.
- 539 [18] D. Wotton, J. Massague, Smad transcriptional corepressors in TGF beta family
540 signaling, *Curr. Top. Microbiol. Immunol.* 254 (2001) 145-164.
- 541 [19] J. Massague, Integration of Smad and MAPK pathways: a link and a linker revisited,
542 *Genes Dev.* 17 (2003) 2993-2997.
- 543 [20] I. Yakymovych, D.P. Ten, C.H. Heldin, S. Souchelnytskyi, Regulation of Smad
544 signaling by protein kinase C, *FASEB J.* 15 (2001) 553-555.
- 545 [21] A.R. Conery, Y. Cao, E.A. Thompson, C.M. Townsend, Jr., T.C. Ko, K. Luo, Akt
546 interacts directly with Smad3 to regulate the sensitivity to TGF-beta induced
547 apoptosis, *Nat. Cell Biol.* 6 (2004) 366-372.
- 548 [22] A. Masszi, C.C. Di, G. Sirokmany, W.T. Arthur, O.D. Rotstein, J. Wang, C.A.
549 McCulloch, L. Rosivall, I. Mucsi, A. Kapus, Central role for Rho in TGF-beta1-
550 induced alpha-smooth muscle actin expression during epithelial-mesenchymal
551 transition, *Am. J. Physiol Renal Physiol* 284 (2003) F911-F924.
- 552 [23] N. Muller, A. Reinacher-Schick, S. Baldus, H.J. van, G. Berx, A. Baar, R.F. van, W.
553 Schmiegell, I. Schwarte-Waldhoff, Smad4 induces the tumor suppressor E-cadherin
554 and P-cadherin in colon carcinoma cells, *Oncogene* 21 (2002) 6049-6058.
- 555 [24] H. Wang, V. Radjendirane, K.K. Wary, S. Chakrabarty, Transforming growth factor
556 beta regulates cell-cell adhesion through extracellular matrix remodeling and

- 557 activation of focal adhesion kinase in human colon carcinoma Moser cells, *Oncogene*
558 23 (2004) 5558-5561.
- 559 [25] V.J. Thannickal, D.Y. Lee, E.S. White, Z. Cui, J.M. Larios, R. Chacon, J.C. Horowitz,
560 R.M. Day, P.E. Thomas, Myofibroblast differentiation by transforming growth factor-
561 beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase, *J.*
562 *Biol. Chem.* 278 (2003) 12384-12389.
- 563 [26] X. Shi-Wen, S.K. Parapuram, D. Pala, Y. Chen, D.E. Carter, M. Eastwood, C.P.
564 Denton, D.J. Abraham, A. Leask, Requirement of transforming growth factor beta-
565 activated kinase 1 for transforming growth factor beta-induced alpha-smooth muscle
566 actin expression and extracellular matrix contraction in fibroblasts, *Arthritis Rheum.*
567 60 (2009) 234-241.
- 568 [27] S. Liu, S.W. Xu, L. Kennedy, D. Pala, Y. Chen, M. Eastwood, D.E. Carter, C.M.
569 Black, D.J. Abraham, A. Leask, FAK is required for TGFbeta-induced JNK
570 phosphorylation in fibroblasts: implications for acquisition of a matrix-remodeling
571 phenotype, *Mol. Biol. Cell* 18 (2007) 2169-2178.
- 572 [28] H. Hayashi, S. Abdollah, Y. Qiu, J. Cai, Y.Y. Xu, B.W. Grinnell, M.A. Richardson,
573 J.N. Topper, M.A. Gimbrone, Jr., J.L. Wrana, D. Falb, The MAD-related protein
574 Smad7 associates with the TGFbeta receptor and functions as an antagonist of
575 TGFbeta signaling, *Cell* 89 (1997) 1165-1173.
- 576 [29] A. Nakao, M. Afrakhte, A. Moren, T. Nakayama, J.L. Christian, R. Heuchel, S. Itoh,
577 M. Kawabata, N.E. Heldin, C.H. Heldin, D.P. Ten, Identification of Smad7, a
578 TGFbeta-inducible antagonist of TGF-beta signalling, *Nature* 389 (1997) 631-635.
- 579 [30] G. Salvador, R. Sanmarti, B. Gil-Torregrosa, A. Garcia-Peiro, J.R. Rodriguez-Cros,
580 J.D. Canete, Synovial vascular patterns and angiogenic factors expression in synovial
581 tissue and serum of patients with rheumatoid arthritis, *Rheumatology. (Oxford)* 45
582 (2006) 966-971.
- 583 [31] S.Y. Kim, S.W. Han, G.W. Kim, J.M. Lee, Y.M. Kang, TGF-beta1 polymorphism
584 determines the progression of joint damage in rheumatoid arthritis, *Scand. J.*
585 *Rheumatol.* 33 (2004) 389-394.
- 586 [32] H. Cheon, S.J. Yu, D.H. Yoo, I.J. Chae, G.G. Song, J. Sohn, Increased expression of
587 pro-inflammatory cytokines and metalloproteinase-1 by TGF-beta1 in synovial
588 fibroblasts from rheumatoid arthritis and normal individuals, *Clin. Exp. Immunol.* 127
589 (2002) 547-552.

- [33] Y. Yamanishi, D.L. Boyle, M. Clark, R.A. Maki, M.D. Tortorella, E.C. Arner, G.S. Firestein, Expression and regulation of aggrecanase in arthritis: the role of TGF-beta, *J. Immunol.* 168 (2002) 1405-1412.
- [34] J.A. Hamilton, D.S. Piccoli, T. Leizer, D.M. Butler, M. Croatto, A.K. Royston, Transforming growth factor beta stimulates urokinase-type plasminogen activator and DNA synthesis, but not prostaglandin E2 production, in human synovial fibroblasts, *Proc. Natl. Acad. Sci. U. S. A* 88 (1991) 7180-7184.
- [35] F. Taketazu, M. Kato, A. Gobl, H. Ichijo, P. ten Dijke, J. Itoh, M. Kyogoku, J. Ronnelid, K. Miyazono, C.H. Heldin, .. Enhanced expression of transforming growth factor-beta s and transforming growth factor-beta type II receptor in the synovial tissues of patients with rheumatoid arthritis, *Lab. Invest.* 70 (1994) 620-630.
- [36] C.Q. Chu, M. Field, E. Abney, R.Q. Zheng, S. Allard, M. Feldmann, R.N. Maini, Transforming growth factor-beta 1 in rheumatoid synovial membrane and cartilage/pannus junction, *Clin. Exp. Immunol.* 86 (1991) 380-386.
- [37] Z. Szekanecz, G.K. Haines, L.A. Harlow, M.R. Shah, T.W. Fong, R. Fu, S.J. Lin, G. Rayan, A.E. Koch, Increased synovial expression of transforming growth factor (TGF)-beta receptor endoglin and TGF-beta 1 in rheumatoid arthritis: possible interactions in the pathogenesis of the disease, *Clin. Immunol. Immunopathol.* 76 (1995) 187-194.
- [38] D.H. Goddard, S.L. Grossman, W.V. Williams, D.B. Weiner, J.L. Gross, K. Eidsvoog, J.R. Dasch, Regulation of synovial cell growth. Coexpression of transforming growth factor beta and basic fibroblast growth factor by cultured synovial cells, *Arthritis Rheum.* 35 (1992) 1296-1303.
- [39] R. Fava, N. Olsen, J. Keski-Oja, H. Moses, T. Pincus, Active and latent forms of transforming growth factor beta activity in synovial effusions, *J. Exp. Med.* 169 (1989) 291-296.
- [40] D. Pohlars, A. Beyer, D. Koczan, T. Wilhelm, H.J. Thiesen, R.W. Kinne, Constitutive upregulation of the transforming growth factor-beta pathway in rheumatoid arthritis synovial fibroblasts, *Arthritis Res. Ther.* 9 (2007) R59.
- [41] A. Müssener, K. Funo, S. Kleinau, L. Klareskog, Dynamic expression of transforming growth factor-betas (TGF-beta) and their type I and type II receptors in the synovial tissue of arthritic rats, *Clin. Exp. Immunol.* 107 (1997) 112-119.

- [42] J.B. Allen, C.L. Manthey, A.R. Hand, K. Ohura, L. Ellingsworth, S.M. Wahl, Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor beta, *J. Exp. Med.* 171 (1990) 231-247.
- [43] S.M. Wahl, J.B. Allen, G.L. Costa, H.L. Wong, J.R. Dasch, Reversal of acute and chronic synovial inflammation by anti-transforming growth factor beta, *J. Exp. Med.* 177 (1993) 225-230.
- [44] Z. Mi, S.C. Ghivizzani, E. Lechman, J.C. Glorioso, C.H. Evans, P.D. Robbins, Adverse effects of adenovirus-mediated gene transfer of human transforming growth factor beta 1 into rabbit knees, *Arthritis Res. Ther.* 5 (2003) R132-R139.
- [45] M. Sakuma, K. Hatsushika, K. Koyama, R. Katoh, T. Ando, Y. Watanabe, M. Wako, M. Kanzaki, S. Takano, H. Sugiyama, Y. Hamada, H. Ogawa, K. Okumura, A. Nakao, TGF-beta type I receptor kinase inhibitor down-regulates rheumatoid synoviocytes and prevents the arthritis induced by type II collagen antibody, *Int. Immunol.* 19 (2007) 117-126.
- [46] M.E. Brandes, J.B. Allen, Y. Ogawa, S.M. Wahl, Transforming growth factor beta 1 suppresses acute and chronic arthritis in experimental animals, *J. Clin. Invest.* 87 (1991) 1108-1113.
- [47] C. Schramm, J. Kriegsmann, M. Protschka, S. Huber, T. Hansen, E. Schmitt, P.R. Galle, M. Blessing, Susceptibility to collagen-induced arthritis is modulated by TGFbeta responsiveness of T cells, *Arthritis Res. Ther.* 6 (2004) R114-R119.
- [48] Y. Chernajovsky, G. Adams, K. Triantaphyllopoulos, M.F. Ledda, O.L. Podhajcer, Pathogenic lymphoid cells engineered to express TGF beta 1 ameliorate disease in a collagen-induced arthritis model, *Gene Ther.* 4 (1997) 553-559.
- [49] F.N. Ziyadeh, K. Sharma, M. Ericksen, G. Wolf, Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-beta, *J. Clin. Invest.* 93 (1994) 536-542.
- [50] F.N. Ziyadeh, Evidence for the involvement of transforming growth factor- in the pathogenesis of diabetic kidney disease: Are Koch's postulates fulfilled?, *Curr. Pract. Med.* 1 (1998) 87-89.
- [51] G. Wolf, K. Sharma, Y. Chen, M. Ericksen, F.N. Ziyadeh, High glucose-induced proliferation in mesangial cells is reversed by autocrine TGF-beta, *Kidney Int.* 42 (1992) 647-656.

- [52] G. Wolf, E. Mueller, R.A. Stahl, F.N. Ziyadeh, Angiotensin II-induced hypertrophy of cultured murine proximal tubular cells is mediated by endogenous transforming growth factor-beta, *J. Clin. Invest.* 92 (1993) 1366-1372.
- [53] T. Yamamoto, T. Nakamura, N.A. Noble, E. Ruoslahti, W.A. Border, Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy, *Proc. Natl. Acad Sci. U. S. A* 90 (1993) 1814-1818.
- [54] K. Sharma, F.N. Ziyadeh, Renal hypertrophy is associated with upregulation of TGF-beta 1 gene expression in diabetic BB rat and NOD mouse, *Am. J. Physiol.* 267 (1994) F1094-01.
- [55] S.W. Hong, M. Isono, S. Chen, Iglesias-de la Cruz MC, D.C. Han, F.N. Ziyadeh, Increased glomerular and tubular expression of transforming growth factor-beta1, its type II receptor, and activation of the Smad signaling pathway in the db/db mouse, *Am. J. Pathol.* 158 (2001) 1653-1663.
- [56] K. Sharma, Y. Jin, J. Guo, F.N. Ziyadeh, Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice, *Diabetes* 45 (1996) 522-530.
- [57] F.N. Ziyadeh, B.B. Hoffman, D.C. Han, Iglesias-de la Cruz MC, S.W. Hong, M. Isono, S. Chen, T.A. McGowan, K. Sharma, Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice, *Proc. Natl. Acad Sci. U. S. A* 97 (2000) 8015-8020.
- [58] M. Isono, A. Mogyrosi, D.C. Han, B.B. Hoffman, F.N. Ziyadeh, Stimulation of TGF-beta type II receptor by high glucose in mouse mesangial cells and in diabetic kidney, *Am. J. Physiol. Renal Physiol.* 278 (2000) F830-F838.
- [59] G. Wolf, G. Zahner, R. Schroeder, R.A. Stahl, Transforming growth factor beta mediates the angiotensin-II-induced stimulation of collagen type IV synthesis in cultured murine proximal tubular cells, *Nephrol. Dial. Transplant.* 11 (1996) 263-269.
- [60] D.C. Han, B.B. Hoffman, S.W. Hong, J. Guo, F.N. Ziyadeh, Therapy with antisense TGF-beta1 oligodeoxynucleotides reduces kidney weight and matrix mRNAs in diabetic mice, *Am. J. Physiol. Renal Physiol.* 278 (2000) F628-F634.
- [61] M. Abbate, C. Zoja, M. Morigi, D. Rottoli, S. Angioletti, S. Tomasoni, C. Zanchi, L. Longaretti, R. Donadelli, G. Remuzzi, Transforming growth factor-beta1 is up-regulated by podocytes in response to excess intraglomerular passage of proteins: a

- central pathway in progressive glomerulosclerosis, *Am. J. Pathol.* 161 (2002) 2179-2193.
- [62] R.E. Gilbert, A. Cox, L.L. Wu, T.J. Allen, U.L. Hulthen, G. Jerums, M.E. Cooper, Expression of transforming growth factor-beta1 and type IV collagen in the renal tubulointerstitium in experimental diabetes: effects of ACE inhibition, *Diabetes* 47 (1998) 414-422.
- [63] G. Wolf, S. Chen, F.N. Ziyadeh, From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy, *Diabetes* 54 (2005) 1626-1634.
- [64] D.T. Wu, M. Bitzer, W. Ju, P. Mundel, E.P. Bottinger, TGF-beta concentration specifies differential signaling profiles of growth arrest/differentiation and apoptosis in podocytes, *J. Am. Soc. Nephrol.* 16 (2005) 3211-3221.
- [65] S.L. Dallas, K. Miyazono, T.M. Skerry, G.R. Mundy, L.F. Bonewald, Dual role for the latent transforming growth factor-beta binding protein in storage of latent TGF-beta in the extracellular matrix and as a structural matrix protein, *J. Cell Biol.* 131 (1995) 539-549.
- [66] M. Isono, S. Chen, S.W. Hong, Iglesias-de la Cruz MC, F.N. Ziyadeh, Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF-beta-induced fibronectin in mesangial cells, *Biochem. Biophys. Res Commun.* 296 (2002) 1356-1365.
- [67] Y. Furuse, N. Hashimoto, M. Maekawa, Y. Toyama, A. Nakao, I. Iwamoto, K. Sakurai, Y. Suzuki, K. Yagui, S. Yuasa, K. Toshimori, Y. Saito, Activation of the Smad pathway in glomeruli from a spontaneously diabetic rat model, OLETF rats, *Nephron Exp. Nephrol.* 98 (2004) e100-e108.
- [68] M. Schiffer, L.E. Schiffer, A. Gupta, A.S. Shaw, I.S. Roberts, P. Mundel, E.P. Bottinger, Inhibitory smads and tgf-Beta signaling in glomerular cells, *J. Am. Soc. Nephrol.* 13 (2002) 2657-2666.
- [69] M. Cordenonsi, M. Montagner, M. Adorno, L. Zacchigna, G. Martello, A. Mamidi, S. Soligo, S. Dupont, S. Piccolo, Integration of TGF-beta and Ras/MAPK signaling through p53 phosphorylation, *Science* 315 (2007) 840-843.
- [70] M. Schiffer, P. Mundel, A.S. Shaw, E.P. Bottinger, A novel role for the adaptor molecule CD2-associated protein in transforming growth factor-beta-induced apoptosis, *J. Biol. Chem.* 279 (2004) 37004-37012.

- [71] K. Yoshioka, T. Takemura, K. Murakami, M. Okada, S. Hino, H. Miyamoto, S. Maki, Transforming growth factor-beta protein and mRNA in glomeruli in normal and diseased human kidneys, *Lab. Invest.* 68 (1993) 154-163.
- [72] T. Yamamoto, N.A. Noble, A.H. Cohen, C.C. Nast, A. Hishida, L.I. Gold, W.A. Border, Expression of transforming growth factor-beta isoforms in human glomerular diseases, *Kidney Int.* 49 (1996) 461-469.
- [73] K. Sharma, F.N. Ziyadeh, B. Alzahabi, T.A. McGowan, S. Kapoor, B.R. Kurnik, P.B. Kurnik, L.S. Weisberg, Increased renal production of transforming growth factor-beta1 in patients with type II diabetes, *Diabetes* 46 (1997) 854-859.
- [74] S. Tsakas, D.S. Goumenos, Accurate measurement and clinical significance of urinary transforming growth factor-beta1, *Am. J. Nephrol.* 26 (2006) 186-193.
- [75] K. Sharma, B.O. Eltayeb, T.A. McGowan, S.R. Dunn, B. Alzahabi, R. Rohde, F.N. Ziyadeh, E.J. Lewis, Captopril-induced reduction of serum levels of transforming growth factor-beta1 correlates with long-term renoprotection in insulin-dependent diabetic patients, *Am. J. Kidney Dis.* 34 (1999) 818-823.
- [76] M.W. Babyatsky, G. Rossiter, D.K. Podolsky, Expression of transforming growth factors alpha and beta in colonic mucosa in inflammatory bowel disease, *Gastroenterology* 110 (1996) 975-984.
- [77] G.C. Blobe, W.P. Schiemann, H.F. Lodish, Role of transforming growth factor beta in human disease, *N. Engl. J. Med.* 342 (2000) 1350-1358.
- [78] A. Izcue, J.L. Coombes, F. Powrie, Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation, *Immunol. Rev.* 212 (2006) 256-271.
- [79] L. Gorelik, S. Constant, R.A. Flavell, Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation, *J. Exp. Med.* 195 (2002) 1499-1505.
- [80] A.B. Kulkarni, C.G. Huh, D. Becker, A. Geiser, M. Lyght, K.C. Flanders, A.B. Roberts, M.B. Sporn, J.M. Ward, S. Karlsson, Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death, *Proc. Natl. Acad. Sci. U. S. A* 90 (1993) 770-774.
- [81] M. Boirivant, I.J. Fuss, A. Chu, W. Strober, Oxazolone colitis: A murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4, *J. Exp. Med.* 188 (1998) 1929-1939.

- 754 [82] M.F. Neurath, S. Pettersson, K.H. Meyer zum Buschenfelde, W. Strober, Local
755 administration of antisense phosphorothioate oligonucleotides to the p65 subunit of
756 NF-kappa B abrogates established experimental colitis in mice, *Nat. Med.* 2 (1996)
757 998-1004.
- 758 [83] F. Powrie, J. Carlino, M.W. Leach, S. Mauze, R.L. Coffman, A critical role for
759 transforming growth factor-beta but not interleukin 4 in the suppression of T helper
760 type 1-mediated colitis by CD45RB(low) CD4+ T cells, *J. Exp. Med.* 183 (1996)
761 2669-2674.
- 762 [84] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, H.L. Weiner, V.K.
763 Kuchroo, Reciprocal developmental pathways for the generation of pathogenic
764 effector TH17 and regulatory T cells, *Nature* 441 (2006) 235-238.
- 765 [85] P.R. Mangan, L.E. Harrington, D.B. O'Quinn, W.S. Helms, D.C. Bullard, C.O. Elson,
766 R.D. Hatton, S.M. Wahl, T.R. Schoeb, C.T. Weaver, Transforming growth factor-beta
767 induces development of the T(H)17 lineage, *Nature* 441 (2006) 231-234.
- 768 [86] M. Veldhoen, R.J. Hocking, C.J. Atkins, R.M. Locksley, B. Stockinger, TGFbeta in
769 the context of an inflammatory cytokine milieu supports de novo differentiation of IL-
770 17-producing T cells, *Immunity* 24 (2006) 179-189.
- 771 [87] V. Santarlasci, L. Maggi, M. Capone, F. Frosali, V. Querci, P.R. De, F. Liotta, L.
772 Cosmi, E. Maggi, S. Romagnani, F. Annunziato, TGF-beta indirectly favors the
773 development of human Th17 cells by inhibiting Th1 cells, *Eur. J. Immunol.* 39 (2009)
774 207-215.
- 775 [88] E. Volpe, N. Servant, R. Zollinger, S.I. Bogiatzi, P. Hupe, E. Barillot, V. Soumelis, A
776 critical function for transforming growth factor-beta, interleukin 23 and
777 proinflammatory cytokines in driving and modulating human T(H)-17 responses, *Nat.*
778 *Immunol.* 9 (2008) 650-657.
- 779 [89] L. Yang, D.E. Anderson, C. Baecher-Allan, W.D. Hastings, E. Bettelli, M. Oukka,
780 V.K. Kuchroo, D.A. Hafler, IL-21 and TGF-beta are required for differentiation of
781 human T(H)17 cells, *Nature* 454 (2008) 350-352.
- 782 [90] X. Yang, J.J. Letterio, R.J. Lechleider, L. Chen, R. Hayman, H. Gu, A.B. Roberts, C.
783 Deng, Targeted disruption of SMAD3 results in impaired mucosal immunity and
784 diminished T cell responsiveness to TGF-beta, *EMBO J.* 18 (1999) 1280-1291.

- 785 [91] G. Monteleone, A. Kumberova, N.M. Croft, C. McKenzie, H.W. Steer, T.T.
786 MacDonald, Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory
787 bowel disease, *J. Clin. Invest* 108 (2001) 601-609.
- 788 [92] N.A. Bhowmick, M. Ghiassi, A. Bakin, M. Aakre, C.A. Lundquist, M.E. Engel, C.L.
789 Arteaga, H.L. Moses, Transforming growth factor-beta1 mediates epithelial to
790 mesenchymal transdifferentiation through a RhoA-dependent mechanism, *Mol. Biol.*
791 *Cell* 12 (2001) 27-36.
- 792 [93] V. Ellenrieder, S.F. Hendler, W. Boeck, T. Seufferlein, A. Menke, C. Ruhland, G.
793 Adler, T.M. Gress, Transforming growth factor beta1 treatment leads to an epithelial-
794 mesenchymal transdifferentiation of pancreatic cancer cells requiring extracellular
795 signal-regulated kinase 2 activation, *Cancer Res.* 61 (2001) 4222-4228.
- 796 [94] K. Fujimoto, H. Sheng, J. Shao, R.D. Beauchamp, Transforming growth factor-beta1
797 promotes invasiveness after cellular transformation with activated Ras in intestinal
798 epithelial cells, *Exp. Cell Res.* 266 (2001) 239-249.
- 799 [95] K. Lehmann, E. Janda, C.E. Pierreux, M. Rytomaa, A. Schulze, M. McMahon, C.S.
800 Hill, H. Beug, J. Downward, Raf induces TGFbeta production while blocking its
801 apoptotic but not invasive responses: a mechanism leading to increased malignancy in
802 epithelial cells, *Genes Dev.* 14 (2000) 2610-2622.
- 803 [96] M. Oft, K.H. Heider, H. Beug, TGFbeta signaling is necessary for carcinoma cell
804 invasiveness and metastasis, *Curr. Biol.* 8 (1998) 1243-1252.
- 805 [97] G. Portella, S.A. Cumming, J. Liddell, W. Cui, H. Ireland, R.J. Akhurst, A. Balmain,
806 Transforming growth factor beta is essential for spindle cell conversion of mouse skin
807 carcinoma in vivo: implications for tumor invasion, *Cell Growth Differ.* 9 (1998) 393-
808 404.
- 809 [98] A.M. Arias, Epithelial mesenchymal interactions in cancer and development, *Cell* 105
810 (2001) 425-431.
- 811 [99] J.P. Thiery, D. Chopin, Epithelial cell plasticity in development and tumor
812 progression, *Cancer Metastasis Rev.* 18 (1999) 31-42.
- 813 [100] R. Kalluri, E.G. Neilson, Epithelial-mesenchymal transition and its implications for
814 fibrosis, *J. Clin. Invest* 112 (2003) 1776-1784.
- 815 [101] S. Saika, S. Kono-Saika, Y. Ohnishi, M. Sato, Y. Muragaki, A. Ooshima, K.C.
816 Flanders, J. Yoo, M. Anzano, C.Y. Liu, W.W. Kao, A.B. Roberts, Smad3 signaling is

- required for epithelial-mesenchymal transition of lens epithelium after injury, *Am. J. Pathol.* 164 (2004) 651-663.
- [102] M. Sato, Y. Muragaki, S. Saika, A.B. Roberts, A. Ooshima, Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction, *J. Clin. Invest* 112 (2003) 1486-1494.
- [103] P.L. Beck, D.K. Podolsky, Growth factors in inflammatory bowel disease, *Inflamm. Bowel. Dis.* 5 (1999) 44-60.
- [104] C. Fiocchi, TGF-beta/Smad signaling defects in inflammatory bowel disease: mechanisms and possible novel therapies for chronic inflammation, *J. Clin. Invest* 108 (2001) 523-526.
- [105] K.B. Hahm, Y.H. Im, T.W. Parks, S.H. Park, S. Markowitz, H.Y. Jung, J. Green, S.J. Kim, Loss of transforming growth factor beta signalling in the intestine contributes to tissue injury in inflammatory bowel disease, *Gut* 49 (2001) 190-198.
- [106] H. Matthes, H. Herbst, D. Schuppan, A. Stallmach, S. Milani, H. Stein, E.O. Riecken, Cellular localization of procollagen gene transcripts in inflammatory bowel diseases, *Gastroenterology* 102 (1992) 431-442.
- [107] A. Stallmach, D. Schuppan, H.H. Riese, H. Matthes, E.O. Riecken, Increased collagen type III synthesis by fibroblasts isolated from strictures of patients with Crohn's disease, *Gastroenterology* 102 (1992) 1920-1929.
- [108] I.C. Lawrance, L. Maxwell, W. Doe, Inflammation location, but not type, determines the increase in TGF-beta1 and IGF-1 expression and collagen deposition in IBD intestine, *Inflamm. Bowel. Dis.* 7 (2001) 16-26.
- [109] J.B. Pucilowska, K.L. Williams, P.K. Lund, Fibrogenesis. IV. Fibrosis and inflammatory bowel disease: cellular mediators and animal models, *Am. J. Physiol Gastrointest. Liver Physiol* 279 (2000) G653-G659.
- [110] L.M. Liem, W.E. Fibbe, H.C. van Houwelingen, E. Goulmy, Serum transforming growth factor-beta1 levels in bone marrow transplant recipients correlate with blood cell counts and chronic graft-versus-host disease, *Transplantation* 67 (1999) 59-65.
- [111] M. Stahle-Backdahl, J. Maim, B. Veress, C. Benoni, K. Bruce, A. Egesten, Increased presence of eosinophilic granulocytes expressing transforming growth factor-beta1 in collagenous colitis, *Scand. J. Gastroenterol.* 35 (2000) 742-746.

- 848 [112] J. Wang, H. Zheng, C.C. Sung, K.K. Richter, M. Hauer-Jensen, Cellular sources of
849 transforming growth factor-beta isoforms in early and chronic radiation enteropathy,
850 *Am. J. Pathol.* 153 (1998) 1531-1540.
- 851 [113] F.F. di Mola, H. Friess, A. Scheuren, S.P. Di, H. Graber, B. Egger, A. Zimmermann,
852 M. Korc, M.W. Buchler, Transforming growth factor-betas and their signaling
853 receptors are coexpressed in Crohn's disease, *Ann. Surg.* 229 (1999) 67-75.
- 854 [114] B.C. McKaig, K. Hughes, P.J. Tighe, Y.R. Mahida, Differential expression of TGF-
855 beta isoforms by normal and inflammatory bowel disease intestinal myofibroblasts,
856 *Am. J. Physiol Cell Physiol* 282 (2002) C172-C182.
- 857 [115] A. Leask, D.J. Abraham, TGF-beta signaling and the fibrotic response, *FASEB J.* 18
858 (2004) 816-827.
- 859 [116] G. Monteleone, B.G. Del Vecchio, I. Monteleone, D. Fina, R. Caruso, V. Gioia, S.
860 Ballerini, G. Federici, S. Bernardini, F. Pallone, T.T. MacDonald, Post-transcriptional
861 regulation of Smad7 in the gut of patients with inflammatory bowel disease,
862 *Gastroenterology* 129 (2005) 1420-1429.
- 863 [117] G. Monteleone, B.G. Del Vecchio, G. Palmieri, P. Vavassori, I. Monteleone, A.
864 Colantoni, S. Battista, L.G. Spagnoli, M. Romano, M. Borrelli, T.T. MacDonald, F.
865 Pallone, Induction and regulation of Smad7 in the gastric mucosa of patients with
866 *Helicobacter pylori* infection, *Gastroenterology* 126 (2004) 674-682.
- 867 [118] K. Randall, J.E. Coggle, Expression of transforming growth factor-beta 1 in mouse
868 skin during the acute phase of radiation damage, *Int. J. Radiat. Biol.* 68 (1995) 301-
869 309.
- 870 [119] K. Randall, J.E. Coggle, Long-term expression of transforming growth factor TGF
871 beta 1 in mouse skin after localized beta-irradiation, *Int. J. Radiat. Biol.* 70 (1996)
872 351-360.
- 873 [120] M. Martin, J.L. Lefaix, P. Pinton, F. Crechet, F. Daburon, Temporal modulation of
874 TGF-beta 1 and beta-actin gene expression in pig skin and muscular fibrosis after
875 ionizing radiation, *Radiat. Res.* 134 (1993) 63-70.
- 876 [121] M. Martin, J. Lefaix, S. Delanian, TGF-beta1 and radiation fibrosis: a master switch
877 and a specific therapeutic target?, *Int. J. Radiat. Oncol. Biol. Phys.* 47 (2000) 277-290.
- 878 [122] S. Schultze-Mosgau, F. Wehrhan, G. Grabenbauer, K. Amann, M. Radespiel-Troger,
879 F.W. Neukam, F. Rodel, Transforming growth factor beta1 and beta2 (TGFbeta2 /

- 880 TGFbeta2) profile changes in previously irradiated free flap beds, *Head Neck* 24
881 (2002) 33-41.
- 882 [123] S. Schultze-Mosgau, F. Wehrhan, F. Rodel, K. Amann, M. Radespiel-Troger, G.G.
883 Grabenbauer, Transforming growth factor-beta receptor-II up-regulation during wound
884 healing in previously irradiated graft beds in vivo, *Wound Repair Regen.* 11 (2003)
885 297-305.
- 886 [124] S. Schultze-Mosgau, M.A. Blaese, G. Grabenbauer, F. Wehrhan, J. Kopp, K. Amann,
887 H.P. Rodemann, F. Rodel, Smad-3 and Smad-7 expression following anti-
888 transforming growth factor beta 1 (TGFbeta1)-treatment in irradiated rat tissue,
889 *Radiother. Oncol.* 70 (2004) 249-259.
- 890 [125] W.A. Border, N.A. Noble, Transforming growth factor beta in tissue fibrosis, *N. Engl.*
891 *J. Med.* 331 (1994) 1286-1292.
- 892 [126] S. Schultze-Mosgau, J. Kopp, M. Thorwarth, F. Rodel, I. Melnychenko, G.G.
893 Grabenbauer, K. Amann, F. Wehrhan, Plasminogen activator inhibitor-I-related
894 regulation of procollagen I (alpha1 and alpha2) by antitransforming growth factor-
895 beta1 treatment during radiation-impaired wound healing, *Int. J. Radiat. Oncol. Biol.*
896 *Phys.* 64 (2006) 280-288.
- 897 [127] D. Ulrich, F. Lichtenegger, M. Eblenkamp, D. Repper, N. Pallua, Matrix
898 metalloproteinases, tissue inhibitors of metalloproteinases, aminoterminal propeptide
899 of procollagen type III, and hyaluronan in sera and tissue of patients with capsular
900 contracture after augmentation with Trilucent breast implants, *Plast Reconstr Surg* 114
901 (2004) 229-236.
- 902 [128] J.A. Schiro, B.M. Chan, W.T. Roswit, P.D. Kassner, A.P. Pentland, M.E. Hemler,
903 A.Z. Eisen, T.S. Kupper, Integrin alpha 2 beta 1 (VLA-2) mediates reorganization and
904 contraction of collagen matrices by human cells, *Cell* 67 (1991) 403-410.
- 905 [129] P.D. Arora, N. Narani, C.A. McCulloch, The compliance of collagen gels regulates
906 transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts,
907 *Am. J. Pathol.* 154 (1999) 871-882.
- 908 [130] M.K. Tibbs, Wound healing following radiation therapy: a review, *Radiother. Oncol.*
909 42 (1997) 99-106.
- 910 [131] R. Tokarek, E.F. Bernstein, F. Sullivan, J. Uitto, J.B. Mitchell, Effect of therapeutic
911 radiation on wound healing, *Clin. Dermatol.* 12 (1994) 57-70.

- [132] S. Schultze-Mosgau, G.G. Grabenbauer, M. Radespiel-Troger, J. Wiltfang, J. Ries, F.W. Neukam, F. Rodel, Vascularization in the transition area between free grafted soft tissues and pre-irradiated graft bed tissues following preoperative radiotherapy in the head and neck region, *Head Neck* 24 (2002) 42-51.
- [133] C. Li, P.B. Wilson, E. Levine, J. Barber, A.L. Stewart, S. Kumar, TGF-beta1 levels in pre-treatment plasma identify breast cancer patients at risk of developing post-radiotherapy fibrosis, *Int. J. Cancer* 84 (1999) 155-159.
- [134] A.M. Feldman, D. McNamara, Myocarditis, *N. Engl. J. Med.* 343 (2000) 1388-1398.
- [135] B. Maisch, A. Richter, A. Sandmoller, I. Portig, S. Pankuweit, Inflammatory dilated cardiomyopathy (DCMI), *Herz* 30 (2005) 535-544.
- [136] L. Rey, V. Lambert, P. Wattre, L. Andreoletti, Detection of enteroviruses ribonucleic acid sequences in endomyocardial tissue from adult patients with chronic dilated cardiomyopathy by a rapid RT-PCR and hybridization assay, *J. Med. Virol.* 64 (2001) 133-140.
- [137] J.L. Melnik, Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses, in: Fields BN, Knipe DM, Howley PM (Eds.), *Virology*, Lippincott-Raven, Philadelphia, 1996, pp. 655-712.
- [138] N.R. Rose, D.A. Neumann, A. Herskovitz, Coxsackievirus myocarditis, in: G.H. Stollerman, J. T. LaMont, J. J. Leonard, M. D. Siperstein (Eds.), *Advances in internal medicine*, Mosby-Year Book, Inc., St.Louis, 1992, pp. 411-429.
- [139] B. Maisch, H. Rupp, [Myocardial fibrosis: a cardiopathophysiologic Janus head], *Herz* 31 (2006) 260-268.
- [140] U. Eismann, M. Sommer, H. Kosmehl, D. Appenroth, C. Fleck, G. Stein, Fibronectin splice variants--prognostic markers for the stage of renal interstitial fibrosis in the rat, *Nephron* 92 (2002) 379-388.
- [141] A. Tamura, S. Kusachi, K. Nogami, A. Yamanishi, Y. Kajikawa, S. Hirohata, T. Tsuji, Tenascin expression in endomyocardial biopsy specimens in patients with dilated cardiomyopathy: distribution along margin of fibrotic lesions, *Heart* 75 (1996) 291-294.
- [142] S. Rosenkranz, M. Flesch, K. Amann, C. Haeuseler, H. Kilter, U. Seeland, K.D. Schluter, M. Bohm, Alterations of beta-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF-beta(1), *Am. J. Physiol. Heart. Circ. Physiol.* 283 (2002) H1253-H1262.

- [143] K. Isoda, Y. Kamezawa, N. Tada, M. Sato, F. Ohsuzu, Myocardial hypertrophy in transgenic mice overexpressing human interleukin 1alpha, *J. Card. Fail.* 7 (2001) 355-364.
- [144] D. Bryant, L. Becker, J. Richardson, J. Shelton, F. Franco, R. Peshock, M. Thompson, B. Giroir, Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha, *Circulation* 97 (1998) 1375-1381.
- [145] T. Kubota, C.F. McTiernan, C.S. Frye, A.J. Demetris, A.M. Feldman, Cardiac-specific overexpression of tumor necrosis factor-alpha causes lethal myocarditis in transgenic mice, *J. Card. Fail.* 3 (1997) 117-124.
- [146] A. Ponten, X. Li, P. Thoren, K. Aase, T. Sjoblom, A. Ostman, U. Eriksson, Transgenic overexpression of platelet-derived growth factor-C in the mouse heart induces cardiac fibrosis, hypertrophy, and dilated cardiomyopathy, *Am. J. Pathol.* 163 (2003) 673-682.
- [147] A. Ponten, E.B. Folestad, K. Pietras, U. Eriksson, Platelet-derived growth factor D induces cardiac fibrosis and proliferation of vascular smooth muscle cells in heart-specific transgenic mice, *Circ. Res.* 97 (2005) 1036-1045.
- [148] B. Gluck, M. Schmidtke, I. Merkle, A. Stelzner, D. Gemsa, Persistent expression of cytokines in the chronic stage of CVB3-induced myocarditis in NMRI mice, *J. Mol. Cell Cardiol.* 33 (2001) 1615-1626.
- [149] M. Schmidtke, B. Gluck, I. Merkle, P. Hofmann, A. Stelzner, D. Gemsa, Cytokine profiles in heart, spleen, and thymus during the acute stage of experimental coxsackievirus B3-induced chronic myocarditis, *J. Med. Virol.* 61 (2000) 518-526.
- [150] T. Shioi, A. Matsumori, S. Sasayama, Persistent expression of cytokine in the chronic stage of viral myocarditis in mice, *Circulation* 94 (1996) 2930-2937.
- [151] M. Satoh, G. Tamura, I. Segawa, A. Tashiro, K. Hiramori, R. Satodate, Expression of cytokine genes and presence of enteroviral genomic RNA in endomyocardial biopsy tissues of myocarditis and dilated cardiomyopathy, *Virchows Arch.* 427 (1996) 503-509.
- [152] Y. Sakata, A.L. Chancey, V.G. Divakaran, K. Sekiguchi, N. Sivasubramanian, D.L. Mann, Transforming growth factor-beta receptor antagonism attenuates myocardial fibrosis in mice with cardiac-restricted overexpression of tumor necrosis factor, *Basic Res. Cardiol.* 103 (2008) 60-68.
- [153] C.H. Heldin, U. Eriksson, A. Ostman, New members of the platelet-derived growth factor family of mitogens, *Arch. Biochem. Biophys.* 398 (2002) 284-290.

- 978 [154] K. Grun, B. Markova, F.D. Bohmer, A. Berndt, H. Kosmehl, C. Leipner, Elevated
979 expression of PDGF-C in coxsackievirus B3-induced chronic myocarditis, *Eur. Heart*
980 *J.* 26 (2005) 728-739.
- 981 [155] R. Wessely, K. Klingel, K.U. Knowlton, R. Kandolf, Cardioslective infection with
982 coxsackievirus B3 requires intact type I interferon signaling: implications for mortality
983 and early viral replication, *Circulation* 103 (2001) 756-761.
- 984 [156] C. Leipner, K. Grun, A. Muller, E. Buchdunger, L. Borsi, H. Kosmehl, A. Berndt, T.
985 Janik, A. Uecker, M. Kiehnopf, F.D. Bohmer, Imatinib mesylate attenuates fibrosis in
986 coxsackievirus b3-induced chronic myocarditis, *Cardiovasc. Res.* 79 (2008) 118-126.
- 987 [157] M. Neef, M. Ledermann, H. Saegesser, V. Schneider, N. Widmer, L.A. Decosterd, B.
988 Rochat, J. Reichen, Oral imatinib treatment reduces early fibrogenesis but does not
989 prevent progression in the long term, *J. Hepatol.* 44 (2006) 167-175.
- 990 [158] R.T. Paniagua, O. Sharpe, P.P. Ho, S.M. Chan, A. Chang, J.P. Higgins, B.H.
991 Tomooka, F.M. Thomas, J.J. Song, S.B. Goodman, D.M. Lee, M.C. Genovese, P.J.
992 Utz, L. Steinman, W.H. Robinson, Selective tyrosine kinase inhibition by imatinib
993 mesylate for the treatment of autoimmune arthritis, *J. Clin. Invest.* 116 (2006) 2633-
994 2642.
- 995 [159] S. Wang, M.C. Wilkes, E.B. Leof, R. Hirschberg, Imatinib mesylate blocks a non-
996 Smad TGF-beta pathway and reduces renal fibrogenesis in vivo, *FASEB J.* 19 (2005)
997 1-11.
- 998 [160] Y. Aono, Y. Nishioka, M. Inayama, M. Ugai, J. Kishi, H. Uehara, K. Izumi, S. Sone,
999 Imatinib as a novel antifibrotic agent in bleomycin-induced pulmonary fibrosis in
1000 mice, *Am. J. Respir. Crit. Care Med.* 171 (2005) 1279-1285.
- 1001 [161] M.C. Wilkes, H. Mitchell, S.G. Penheiter, J.J. Dore, K. Suzuki, M. Edens, D.K.
1002 Sharma, R.E. Pagano, E.B. Leof, Transforming growth factor-beta activation of
1003 phosphatidylinositol 3-kinase is independent of Smad2 and Smad3 and regulates
1004 fibroblast responses via p21-activated kinase-2, *Cancer Res.* 65 (2005) 10431-10440.
- 1005 [162] C.E. Daniels, M.C. Wilkes, M. Edens, T.J. Kottom, S.J. Murphy, A.H. Limper, E.B.
1006 Leof, Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents
1007 bleomycin-mediated lung fibrosis, *J. Clin. Invest.* 114 (2004) 1308-1316.
- 1008 [163] R. Plattner, L. Kadlec, K.A. DeMali, A. Kazlauskas, A.M. Pendergast, c-Abl is
1009 activated by growth factors and Src family kinases and has a role in the cellular
1010 response to PDGF, *Genes Dev.* 13 (1999) 2400-2411.

- 1011 [164] U. Seeland, C. Haeuseler, R. Hinrichs, S. Rosenkranz, T. Pfitzner, K. Scharffetter-
1012 Kochanek, M. Bohm, Myocardial fibrosis in transforming growth factor-beta(1) (TGF-
1013 beta(1)) transgenic mice is associated with inhibition of interstitial collagenase, *Eur. J.*
1014 *Clin. Invest.* 32 (2002) 295-303.
- 1015 [165] H. Xiao, Y.Y. Zhang, Understanding the role of transforming growth factor-beta
1016 signalling in the heart: overview of studies using genetic mouse models, *Clin. Exp.*
1017 *Pharmacol. Physiol.* 35 (2008) 335-341.
- 1018 [166] K.J. Gordon, G.C. Blobe, Role of transforming growth factor-beta superfamily
1019 signaling pathways in human disease, *Biochim. Biophys Acta* 1782 (2008) 197-228.
- 1020 [167] A.L. Mead, T.T. Wong, M.F. Cordeiro, I.K. Anderson, P.T. Khaw, Evaluation of anti-
1021 TGF-beta2 antibody as a new postoperative anti-scarring agent in glaucoma surgery,
1022 *Invest. Ophthalmol. Vis. Sci.* 44 (2003) 3394-3401.
- 1023 [168] C.P. Denton, P.A. Merkel, D.E. Furst, D. Khanna, P. Emery, V.M. Hsu, N. Silliman, J.
1024 Streisand, J. Powell, A. Akesson, J. Coppock, F. Hoogen, A. Herrick, M.D. Mayes, D.
1025 Veale, J. Haas, S. Ledbetter, J.H. Korn, C.M. Black, J.R. Seibold, Recombinant human
1026 anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a
1027 multicenter, randomized, placebo-controlled phase I/II trial of CAT-192, *Arthritis*
1028 *Rheum.* 56 (2007) 323-333.
- 1029 [169] [<http://clinicaltrials.gov/ct2/show/NCT00125385>]
- 1030 [170] [<http://clinicaltrials.gov/ct2/show/NCT00464321>]
- 1031 [171] [<http://clinicaltrials.gov/ct2/show/NCT00356460>]
- 1032 [172] P. Juarez, M.M. Vilchis-Landeros, J. Ponce-Coria, V. Mendoza, R. Hernandez-Pando,
1033 N.A. Bobadilla, F. Lopez-Casillas, Soluble betaglycan reduces renal damage
1034 progression in db/db mice, *Am. J. Physiol. Renal. Physiol.* 292 (2007) F321-F329.
- 1035 [173] M.A. Rowland-Goldsmith, H. Maruyama, K. Matsuda, T. Idezawa, M. Ralli, S. Ralli,
1036 M. Korc, Soluble type II transforming growth factor-beta receptor attenuates
1037 expression of metastasis-associated genes and suppresses pancreatic cancer cell
1038 metastasis, *Mol. Cancer Ther.* 1 (2002) 161-167.
- 1039 [174] R.S. Muraoka, N. Dumont, C.A. Ritter, T.C. Dugger, D.M. Brantley, J. Chen, E.
1040 Easterly, L.R. Roebuck, S. Ryan, P.J. Gotwals, V. Koteliansky, C.L. Arteaga,
1041 Blockade of TGF-beta inhibits mammary tumor cell viability, migration, and
1042 metastases, *J. Clin. Invest.* 109 (2002) 1551-1559.

- [175] P. Hau, P. Jachimczak, R. Schlingensiepen, F. Schulmeyer, T. Jauch, A. Steinbrecher, A. Brawanski, M. Proescholdt, J. Schlaier, J. Buchroithner, J. Pichler, G. Wurm, M. Mehdorn, R. Strege, G. Schuierer, V. Villarrubia, F. Fellner, O. Jansen, T. Straube, V. Nohria, M. Goldbrunner, M. Kunst, S. Schmaus, G. Stauder, U. Bogdahn, K.H. Schlingensiepen, Inhibition of TGF-beta2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies, *Oligonucleotides* 17 (2007) 201-212.
- [176] J. Nemunaitis, R.O. Dillman, P.O. Schwarzenberger, N. Senzer, C. Cunningham, J. Cutler, A. Tong, P. Kumar, B. Pappen, C. Hamilton, E. DeVol, P.B. Maples, L. Liu, T. Chamberlin, D.L. Shawler, H. Fakhrai, Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer, *J. Clin. Oncol.* 24 (2006) 4721-4730.
- [177] E. Suzuki, S. Kim, H.K. Cheung, M.J. Corbley, X. Zhang, L. Sun, F. Shan, J. Singh, W.C. Lee, S.M. Albelda, L.E. Ling, A novel small-molecule inhibitor of transforming growth factor beta type I receptor kinase (SM16) inhibits murine mesothelioma tumor growth in vivo and prevents tumor recurrence after surgical resection, *Cancer Res* 67 (2007) 2351-2359.
- [178] N.G. Frangogiannis, G. Ren, O. Dewald, P. Zymek, S. Haudek, A. Koerting, K. Winkelmann, L.H. Michael, J. Lawler, M.L. Entman, Critical role of endogenous thrombospondin-1 in preventing expansion of healing myocardial infarcts, *Circulation* 111 (2005) 2935-2942.
- [179] Chuva de Sousa Lopes SM, A. Feijen, J. Korving, O. Korchynskyi, J. Larsson, S. Karlsson, P. ten Dijke, K.M. Lyons, R. Goldschmeding, P. Doevendans, C.L. Mummery, Connective tissue growth factor expression and Smad signaling during mouse heart development and myocardial infarction, *Dev. Dyn.* 231 (2004) 542-550.
- [180] I. Gotis-Graham, P.J. Hogg, H.P. McNeil, Significant correlation between thrombospondin 1 and serine proteinase expression in rheumatoid synovium, *Arthritis Rheum.* 40 (1997) 1780-1787.
- [181] F. Wu, T. Dassopoulos, L. Cope, A. Maitra, S.R. Brant, M.L. Harris, T.M. Bayless, G. Parmigiani, S. Chakravarti, Genome-wide gene expression differences in Crohn's disease and ulcerative colitis from endoscopic pinch biopsies: insights into distinctive pathogenesis, *Inflamm. Bowel. Dis.* 13 (2007) 807-821.
- [182] N.A. Wahab, L. Schaefer, B.S. Weston, O. Yiannikouris, A. Wright, A. Babelova, R. Schaefer, R.M. Mason, Glomerular expression of thrombospondin-1, transforming

- growth factor beta and connective tissue growth factor at different stages of diabetic nephropathy and their interdependent roles in mesangial response to diabetic stimuli, *Diabetologia* 48 (2005) 2650-2660.
- [183] F.M. Brennan, D. Chantry, M. Turner, B. Foxwell, R. Maini, M. Feldmann, Detection of transforming growth factor-beta in rheumatoid arthritis synovial tissue: lack of effect on spontaneous cytokine production in joint cell cultures, *Clin. Exp. Immunol.* 81 (1990) 278-285.
- [184] J. Hao, H. Ju, S. Zhao, A. Junaid, T. Scammell-La Fleur, I.M. Dixon, Elevation of expression of Smads 2, 3, and 4, decorin and TGF-beta in the chronic phase of myocardial infarct scar healing, *J Mol. Cell Cardiol.* 31 (1999) 667-678.
- [185] J. Waltenberger, L. Lundin, K. Oberg, E. Wilander, K. Miyazono, C.H. Heldin, K. Funai, Involvement of transforming growth factor-beta in the formation of fibrotic lesions in carcinoid heart disease, *Am J Pathol* 142 (1993) 71-78.
- [186] S. Klahr, Mechanisms of progression of chronic renal damage, *J. Nephrol.* 12 Suppl 2 (1999) S53-S62.
- [187] S. Schultze-Mosgau, F. Wehrhan, K. Amann, M. Radespiel-Troger, F. Rodel, G.G. Grabenbauer, In Vivo TGF-beta 3 expression during wound healing in irradiated tissue. An experimental study, *Strahlenther. Onkol.* 179 (2003) 410-416.
- [188] S. Schultze-Mosgau, F. Wehrhan, F. Rodel, K. Amann, M. Radespiel-Troger, G.G. Grabenbauer, Transforming growth factor-beta receptor-II up-regulation during wound healing in previously irradiated graft beds in vivo, *Wound Repair Regen.* 11 (2003) 297-305.

Table 1. Expression of TGF- β related proteins in human disease compared to controls (normal or inflammatory)

| Organ | Irradiated skin | Heart | Synovial membrane | | Gut | | Kidney |
|---------------------|-----------------|--------------------------|------------------------------|---------------|-------------|---------------|----------------------|
| Disease | Wound healing | Fibrotic heart diseases* | RA | CD inflamed | CD fibrotic | UC | Diabetic nephropathy |
| Molecule | | | | | | | |
| THBS1 | n.d. | ↑[178,179] | ↑[40,180] | ↓[181] | n.d. | ↔[181] | ↑[182] |
| Latent-TGF- β | ↑[122] | n.d. | ↔[183], ↑[39] | n.d. | n.d. | n.d. | ↑[182] |
| TGF- β 1 | ↑[122] | ↑[184,185] | ↔[35,36,40,183], ↑[37,38] | ↑[76,108,114] | ↑[108,114] | ↑[76,108,114] | ↔[186], ↑[53,72] |
| TGF- β 2 | ↑[122] | ↔[185] | ↔[35,183] | ↑[114] | ↑[114] | ↔[114] | ↑[72] |
| TGF- β 3 | ↓[187] | ↑[185] | ↔[35,183] | ↓[114] | ↓[114] | ↔[114] | ↔[186], ↑[72] |
| TGFBR1 | n.d. | ↓[184] | ↑[40] | ↔[113] | n.d. | n.d. | n.d. |
| TGFBR2 | ↑[188] | ↔[184] | ↑[35,183] | ↑[113] | n.d. | n.d. | n.d. |

↑ increased, ↓ decreased, ↔ no change, **n.d.** not determined; *mostly following myocardial infarction

FIGURE LEGENDS

Figure 1: TGF- β 1 induces different, but overlapping response in different organ systems.

Figure 2: Molecules of the TGF- β -pathway ranging from the release of TGF- β , binding of receptors, signaling through Smads, FAK and MAPK to its entry into the nucleus and the regulation of the transcriptional activity (adapted from [40]).

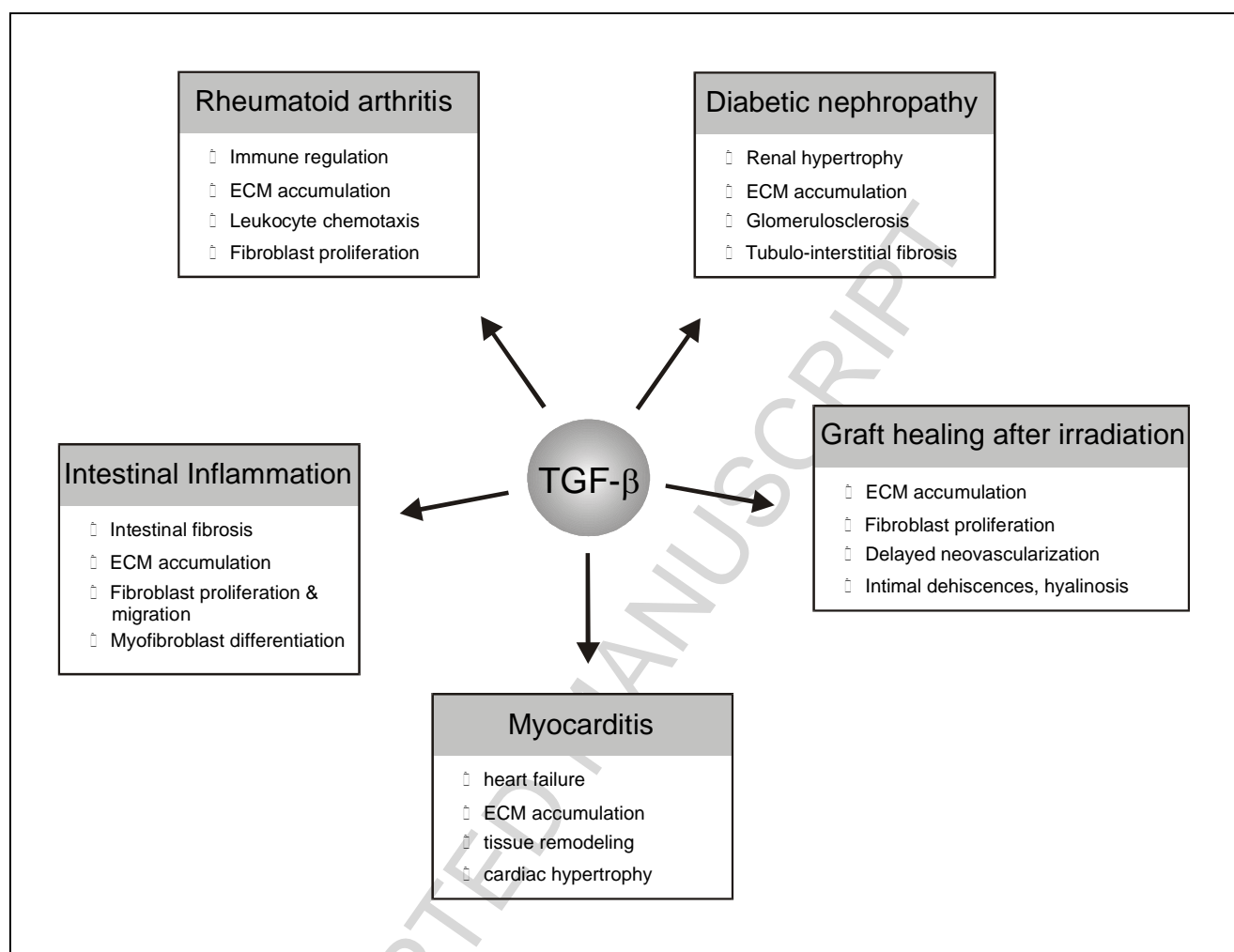


Figure 1

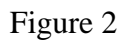


Figure 2