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EDITORIAL

# TGF-β in aging and disease

**K. Krieglstein · K. Miyazono · P. ten Dijke · K. Unsicker**

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## Introduction

The superfamily of transforming growth factor-βs (TGF-βs) constitutes one of the most versatile families of signaling molecules, being involved in a broad range of biological processes spanning from development, cell death and maintenance, to aging and disease. The field has made impressive progress during the past decade. Novel signaling pathways and contextual mechanisms have been discovered. New insights into the roles of TGF-βs in tumor initiation and progression have been achieved. Numerous associations of TGF-βs with various diseases have been newly discovered or elucidated in much more detail than before, including atherosclerosis, acute and chronic liver and kidney disease, osteoarthritis and neurodegenerative diseases.

This Special Issue highlights several of the recent advances in the TGF-β field. The purpose of this collection of reviews is not to present a comprehensive overview of the current status of knowledge on TGF-βs. Rather, it is the guest editors' concept to focus on recent progress seen from the perspective of dynamic cell performances, signaling and complex disease states.

K. Krieglstein (✉) · K. Unsicker
Department of Molecular Embryology,
Institute of Anatomy and Cell Biology, University of Freiburg,
Albertstrasse 17,
79104 Freiburg, Germany
e-mail: kerstin.krieglstein@anat.uni-freiburg.de

K. Miyazono
Department of Molecular Pathology,
Graduate School of Medicine, University of Tokyo,
Tokyo 113-0033, Japan

P. ten Dijke
Department of Molecular Cell Biology and Centre for Biomedical Genetics, Leiden University Medical Center,
Postbus 9600, 2300 RC Leiden, The Netherlands

## A brief overview of articles in this Special Issue

An initial series of articles in this Special Issue reviews canonical and non-canonical TGF-β signaling. Heldin and Moustakas (2011) focus on the role of Smads and the canonical TGF-β signaling pathway. Important intracellular mediators of TGF-β signaling are members of the Smad family. Smad2 and 3 are activated by C-terminal receptor-mediated phosphorylation. They establish complexes with Smad4 and are translocated to the nucleus to regulate transcription, by cooperating with other transcription factors, co-activators and co-repressors. Smads have important roles in the execution of TGF-β-induced processes resulting in, for example, cell growth arrest and epithelial-mesenchymal transition (EMT). The activity and stability of Smad molecules are regulated by numerous post-translational modifications, including phosphorylation, ubiquitination, sumoylation, acetylation and poly(ADP)-ribosylation. Perturbations of Smad functions are key events in certain diseases such as cancer.

Mu et al. (2011) review and discuss non-Smad signaling pathways activated by the TGF-β receptors. These include, for example, the p38 and c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) pathways. Ubiquitin ligase tumor necrosis factor (TNF)-receptor-associated factor 6 (TRAF6) and TGFβ-associated kinase 1 (TAK1) have recently been shown to be crucial for the activation of the p38 and JNK MAPK pathways. The phosphoinositide 3-kinase-Akt-mTOR pathway, the small GTPases Rho, Rac, and Cdc42 and the Ras-Erk-MAPK pathway constitute other non-Smad signaling pathways. Specification in terms of subcellular localization, activity and duration of the signal is mediated by, for example, post-translational modifications of the signaling components.

The context-dependent effects of TGF-βs have long been an enigma in understanding the biology of TGF-β. Ikushima and Miyazono (2011) review established data and

discuss novel data related to this classical question in the field of research on TGF-β signaling. Cross-interaction with other signaling pathways, different repertoires of Smad-binding transcription factors and genetic alterations, especially in cancer cells, provide just a few answers to the question. Recently, epigenetic regulation and non-coding RNAs have been recognized as mechanisms involved in context-dependent effects of TGF-β.

c-Ski is an evolutionary conserved protein that has been implicated in diverse cellular processes such as proliferation, differentiation, transformation and tumor progression. Bonnon and Atanasoski (2011) review mechanisms by which c-Ski regulates essential signaling pathways, e.g., the TGF-β pathway. They summarize the diverse roles attributed to c-Ski during normal development and in cancer progression and discuss future strategies to unravel further the complex nature of c-Ski actions in a context-dependent manner.

Spittau and Krieglstein (2011) describe two members of the Sp1/Krüppel-like zinc finger transcription factors, namely Klf10 and Klf11 and their role as mediators of TGF-β signaling. Klf10 and Klf11 are now recognized as being upregulated not only by TGF-β but by many other growth factors and by cytokines and hormones. The focus of the review is on the transcriptional regulation of Klf10 and Klf11, their involvement in the regulation of the TGF-β signaling pathway and their possible role as molecules mediating crosstalks between various signaling pathways. The article also provides an overview of the pro-apoptotic and anti-proliferative functions of Klf10 and Klf11.

The following articles focus on the roles of TGF-βs in tumor biology, mechanisms underlying the growth inhibitory effects of TGF-β and the promotion of oncogenic activity by TGF-βs at advanced stages. Zu et al. (2011) provide an update on the current understanding of TGF-β signaling in cancer development and its progression focusing on breast cancer. They also review the current approaches of TGF-β signaling-targeted therapeutics for human malignancies.

TGF-β-induced EMT during cancer progression is the topic of the article by Wendt et al. (2011). Increasing evidence suggests that prior to and during metastatic progression, a functional switch occurs in the oncogenic activity of TGF-β known as the "TGF-β paradox". The molecular determinants governing the TGF-β paradox are complex. Recent findings link genetic and epigenetic events, together with alterations within the tumor environment, to the acquisition of oncogenic activity by TGF-β. These events contribute to endowing TGF-β with the capacity to direct metastatic progression via EMT. As a result, carcinoma cells abandon their polarization and adopt mesenchymal-like apolar phenotypes. Numerous signaling molecules, transcription factors and microRNAs operate in mediating the initiation and resolution of this complex transdifferentiation event. In addition to its ability to enhance carcinoma cell invasion and metastasis, EMT also invests transitioned cells with stem-like properties, including the acquisition of self-renewal and tumor-initiating capabilities coupled to chemoresistance.

In addition to tumor progression, EMT is also important in the development of organ fibrosis, including renal fibrosis. Carew and associates (2011) review the three subtypes (type I, II and III) into which EMT has been classified and the functional consequences. They highlight findings concerning type II EMT as a direct contributor to the kidney myofibroblast population in the development of renal fibrosis. They describe type II EMT in diabetic nephropathy, including the signaling molecules, pathways involved and implications of specific microRNA. They summarize new insights into the activation and development of EMT during disease processes, insights that might lead to possible therapeutic interventions to suppress EMTs and potentially reverse organ fibrosis.

The role of TGF-β in renal fibrosis is also discussed in the article by Samarakoon and co-workers (2011) focusing on unilateral ureteral obstruction as a useful model. Regardless of etiology, elevated TGF-β1 levels are causatively linked to the activation of profibrotic signaling pathways initiated by angiotensin, glucose and oxidative stress. Plasminogen activator inhibitor-1 (PAI-1), a major effector and downstream target of TGF-β1 in the progression of several clinically important fibrotic disorders, is highly up-regulated in this model. Smad and non-Smad pathways including pp60c-src, epidermal growth factor receptor, MAPK and p53 are required for PAI-1 induction by TGF-β1. The authors summarize the molecular basis and translational significance of TGF-β1-stimulated PAI-1 in the progression of kidney disease and the relevance of these findings for tissue fibrosis in general.

Staying with kidney pathophysiology, Lee (2011) reviews recent advances in understanding the role of TGF-β in progressive podocyte disease. Mechanical pressure or biomechanical strain in podocytopathies can cause the overexpression of TGF-β and angiotensin II (Ang II). Oxidative stress induced by Ang II might activate the latent TGF-β, which then activates Smads and Ras-Erk signaling pathways in podocytes. As a result of enhanced TGF-β activity in podocytes, the thickness of the glomerular basement membrane is increased via the overproduction of basement membrane proteins and impaired degradation. Podocytes can apoptose, detach from the basement membrane and undergo EMT. Furthermore, activated TGF-β/Smad signaling by podocytes might induce connective tissue growth factor and vascular endothelial growth factor overexpression, which could act as a paracrine effector mechanism on mesangial cells to stimulate mesangial matrix synthesis.

TGF-β in chronic kidney disease is also the topic of the article byLópez-Hernandez and López-Novoa (2011). Gomerular fibrosis glomerulosclerosis is a major cause of

glomerular filtration rate reduction in chronic kidney disease and all three major glomerular cell types (podocytes or visceral epithelial cells, mesangial cells and endothelial cells) participate in the fibrotic process. In addition to triggering podocyte apoptosis and detachment from the glomerular basement membrane, mesangial expansion and EMT, TGF-β has also been shown to mediate several key tubular pathological events, such as fibroblast proliferation, tubular and fibroblast extracellular matrix (ECM) production and epithelial cell death.

Perturbation of TGF-β signaling by pro-inflammatory cytokines in hepatocytes promotes both fibrogenesis and carcinogenesis (fibro-carcinogenesis). This topic is addressed by Matsuzaki (2011) in this Special Issue. Recent detailed analysis of the TGF-β signaling process has significantly expanded insights into fibro-carcinogenic effects on chronically damaged hepatocytes. TGF-β type I receptor and pro-inflammatory cytokine-activated kinases differentially phosphorylate Smad2 and Smad3 to create phosphoisoforms phosphorylated at the C-terminal (C), linker (L), or both (L/C) regions. After acute liver injury, TGF-β-mediated pSmad3C signaling terminates hepatocytic proliferation induced by the pro-inflammatory cytokine-mediated mitogenic pSmad3L pathway and TGF-β and pro-inflammatory cytokines synergistically enhance collagen synthesis by activated hepatic stellate cells via pSmad2L/C and pSmad3L/C pathways. During chronic liver disease progression, pre-neoplastic hepatocytes persistently affected by TGF-β together with pro-inflammatory cytokines come to exhibit the same carcinogenic (mitogenic) pSmad3L and fibrogenic pSmad2L/C signaling, thereby accelerating liver fibrosis while increasing the risk of hepatocellular carcinoma.

Dooley and ten Dijke (2011) also address TGF-β as a central regulator in chronic liver disease; TGF-β contributes to all stages of disease progression from initial liver injury, through inflammation and fibrosis, to cirrhosis and hepatocellular carcinoma. Liver-damage-induced levels of active TGF-β enhance hepatocyte destruction and mediate hepatic stellate cell and fibroblast activation resulting in a wound-healing response, including myofibroblast generation and ECM deposition. Interference with TGF-β signaling in various short-term animal models has demonstrated promising results. However, liver disease progression in human is a process that takes decades with various phases in which TGF-β or its targeting might have both beneficial and adverse outcomes. Based on state-of-the-art literature, the authors summarize the cell-type-directed double-edged role of TGF-β in the different liver disease stages.

Osteoarthritis (OA) is a disease of articular cartilage in which changes in chondrocytes lead to the autolytic destruction of the cartilage. In articular cartilage, TGF-β has recently been found to signal not only via activin receptor-like kinase 5 (ALK5)-induced Smad2/3 phosphorylation but also via ALK1-induced Smad1/5/8 phosphorylation. As reviewed by van der Kraan et al. (2011) in aging cartilage and experimental OA, the ratio ALK1/ALK5 has been found to be increased and the expression of ALK1 is correlated with matrix metalloproteinase-13 expression. The authors discuss the possibility that an age-dependent shift toward Smad1/5/8 signaling might trigger the differentiation of articular chondrocytes with an autolytic phenotype.

Similar to organ fibrosis, TGF-β also has important implications for progressive vascular fibrosis and atherosclerotic lesions. In their review, Toma and McCaffrey (2011) discuss the role of TGF-β as a major orchestrator of the vascular fibroproliferative response. In the early stages of repair, TGF-β is released from platelets and activated from matrix reservoirs. Subsequently, it stimulates the chemotaxis of repair cells, modulates immunity and inflammation and induces matrix production. At later stages, it negatively regulates fibrosis. TGF-β might also be important in arterial calcification. Many of the effects of TGF-β are essential to normal tissue repair and thus, TGF-β is often thought to be "atheroprotective". Collectively, this article provides a survey of the many component pathways involved in atherogenesis.

Similar to EMT, endothelial-to-mesenchymal transition (EndMT) converts endothelial cells to a more mesenchymal cell type, which can give rise not only to fibroblasts but also to bone cells; van Meeteren and ten Dijke (2011) review the essential molecular and cellular features of this process, which is not only essential during embryonic development and tissue regeneration but also under pathological conditions such as organ fibrosis. EndMT also contributes to the generation of cancer-associated fibroblasts. Like EMT, EndMT can be induced by TGF-β. Many studies have suggested the important role of TGF-β receptor/Smad signaling and downstream targets, such as Snail transcriptional repressor in EndMT. By selective targeting of TGF-β receptor signaling, pathological EndMT might be inhibited for the therapeutic benefit of patients with cancer and fibrosis.

The TGF-β signaling network in cardiovascular damage and repair is the topic of the review by Doetschman et al. (2011). The majority of children with congenital heart disease now live into adulthood because of remarkable surgical and medical advances. These patients currently represent the largest age group with adult cardiovascular diseases. They include patients with heart diseases that were not detected or not treated during childhood, those whose defects were surgically corrected but now need revision, those with exercise problems and those with age-related degenerative diseases. Cardiovascular diseases in this population are relatively new and are not well understood. Since there is a developmental basis to adult

cardiovascular disease, TGF-β signaling pathways that are essential for proper cardiovascular development might also play critical roles in the homeostatic, repair and stress response processes involved in adult cardiovascular diseases. This review summarizes current information on a subset of TGF-β ligand and receptor genes and related effector genes that, when dysregulated, are known to lead to cardiovascular diseases and adult cardiovascular deficiencies. This knowledge might also contribute to the development of therapeutic approaches for these diseases.

Aortic aneurysm is predominantly found in the ascending aorta in Marfan syndrome (MFS) patients. However, descending aortic disease has emerged as a problem, since people are living longer because of the improved medical and surgical management of the ascending aorta. Mechanisms involved in the transition of normal aortic tissue to aneurysm have largely remained unclear. In this original article, Haskett and coworkers (2011) have determined the signs of descending aortic disease before disease onset in Fbn1+/C1039G mice, a validated mouse model of disease susceptibility and progression of aortic aneurysm of MFS. They have analyzed tubular unfixed non-aneurysmal descending thoracic aorta from wild-type and Fbn1+/C1039G mice. Fbn1+/C1039G mouse aorta is more compliant in the circumferential direction. Two-photon imaging shows the defective organization of adventitial collagen fibers in the pressurized aortas of Fbn1+/C1039G mice. Additionally, disruption in the elastic lamina has been noted in the absence of aneurysm in the pressurized aortas but not in unpressurized aortas of Fbn1+/C1039G mice. At the molecular level, this altered tissue behavior in the non-aneurysmal descending aortas of Fbn1+/C1039G mice is accompanied by an increasing trend of Smad but not non-Smad, signaling. The ability to reveal the presence of altered biomechanics and microstructure coupled with subtle changes in TGF-β signaling provides a novel surrogate measure of tissue susceptibility to aneurysm before disease onset.

The proper control of the vascular perfusion of the brain is crucial for the generation and maintenance of neuronal and glial cell functions. Beck and Schachtrup (2011) present an intriguing mechanism by which TGF-β is activated subsequent to brain vascular damage. The blood–brain barrier (BBB), which is formed by vascular cells and glia, separates components of the circulating blood from neurons. Blood components immediately leak into the brain after mechanical damage or as a consequence of a compromised BBB in brain disease thereby changing the extracellular environment at sites of vascular damage. Fibrinogen, which is deposited in the nervous system promptly after vascular damage, acts as an initial scar inducer by promoting the availability of active TGF-β. Fibrinogen-bound latent TGF-β interacts with astrocytes, leading to active TGF-β formation and the activation of the TGF-β/Smad signaling pathway. Active TGF-β generated at the neurovascular interface exerts pleiotropic effects. The authors describe scenarios in which these effects might contribute to degeneration and regeneration processes. Elucidation of the molecular details of these processes might also open avenues for new therapeutic approaches.

Primary open-angle glaucoma (POAG) is a major cause of blindness worldwide and is associated with elevated levels of TGF-β2 in the aqueous humor and reactive optic nerve astrocytes. As reviewed by Fuchshofer and Tamm (2011), TGF-β2 is a key player contributing to the structural changes that are characteristically seen in POAG with regard to the ECM of the trabecular meshwork and optic nerve head. The changes are remarkably similar in trabecular meshwork cells and optic nerve head astrocytes. A possible scenario involves ECM changes in the trabecular meshwork leading to an increase of aqueous humor outflow resistance thereby causing higher intraocular pressure (IOP). In the optic nerve head, TGF-β2-induced changes might contribute to the deformation of optic nerve axons causing an impairment of axonal transport and neurotrophic supply. In addition, high IOP might induce the expression of activated TGF-β1 in trabecular meshwork cells and optic nerve head astrocytes, an event that again might significantly lead to the progress of axonal degeneration. The authors describe molecular details of these processes including the downstream targets of TGF-β1 and -β2, the blocking effects of BMP-4 and BMP-7 and of gremlin that inhibits BMP signaling and several species of microRNAs.

Alzheimer's disease (AD) is a neurodegenerative disorder that affects about 35 million people worldwide. At a cellular level, β-amyloid (Aβ) plaques, neurofibrillary tangles and neuronal loss characterize AD. As reviewed by Caraci et al. (2011), an impairment of the TGF-β1 signaling pathway has recently been demonstrated to be specific to the AD brain and, particularly, to the early phase of the disease. The deficiency of TGF-β1 signaling is associated with Aβ pathology and neurofibrillary tangle formation in AD animal models. Reduced TGF-β1 signaling seems to contribute to several pathopysiological hallmarks of AD including microglial activation. The authors discuss the possibility that based on the neuroprotective effect of TGF-β, the rescue of TGF-β signaling might slow down the neurodegenerative process in AD.

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