

The complex regulation of TGF- β in cardiovascular disease

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Abstract: Transforming growth factor β (TGF- β 1) is a pleiotropic cytokine with many and complex effects in cell and tissue physiology. This is made possible by a very complex and interwoven signaling system, whose regulation continues to be the focus of a growing line of research. This complex regulation translates to a key role in cardiovascular physiology, hemostasis, and the blood–vessel interface. In accordance with this, the TGF- β 1 pathway appears to be deregulated in related disorders, such as atherosclerotic vascular disease and myeloproliferative syndromes. It is expected that the growing amount of experimental and clinical research will yield medical advances in the applications of knowledge of the TGF- β 1 pathway to diagnosis and therapeutics.

Keywords: transforming growth factor beta, pathway, Smads, non-Smads, atherosclerosis, myeloproliferative syndromes

Introduction

Transforming growth factor β (TGF- β 1) is a pleiotropic cytokine, which has been demonstrated to regulate a wide array of biological processes. It plays a major role in the regulation of vascular function and hemostasis. Therefore, it can be considered as a putative therapeutic target in disorders of the blood–vessel interface, such as atherosclerosis and myeloproliferative syndromes. The present narrative review highlights the most important advances in the knowledge of TGF- β regulation in cardiovascular disease. This review has been prepared after a comprehensive search through MEDLINE. Search terms were “TGF beta”, “cardiovascular”, “atherosclerosis”, “myeloproliferative syndromes”, “pathway”, and “regulation”. A broader coverage of research strategy can be found in Gasparyan et al.¹

Regulation of TGF- β 1 physiology

The canonical TGF- β 1 pathway

The regulation of TGF- β 1 is shown in Figure 1. Briefly, active TGF- β 1 is released from its latency-associated peptide by activating proteases. Then, it binds to the TGF- β -RII, which acts as a Ser/Thre kinase.² This Ser/Thre kinase activity phosphorylates TGF- β -RI which may be present in several isoforms termed activin-like kinases (ALKs).³ In general, TGF- β 1 stimulates ALK-5 and phosphorylates second messenger proteins termed Smads.⁴ Smad2 or Smad3 form a heterodimer with Smad4, and internalize into the nucleus to decrease the proliferation/apoptosis ratio,⁵ increase differentiation,⁶ and inhibit the expression of inflammatory molecules.⁷

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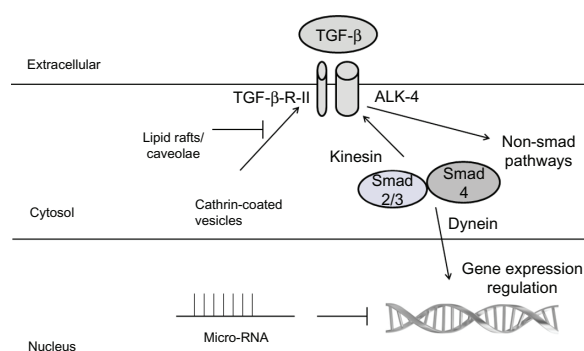


Figure 1 Summary of the main regulators of the TGF- β pathway.

Notes: Briefly, TGF- β binds to receptor type 2 and phosphorylates the type 1 receptor, whose main isoform is termed ALK-4 (activin-like kinase). This kinase phosphorylates and activates Smad2 or Smad3, which forms a heterodimer with Smad4, and internalizes into the nucleus to regulate gene expression. TGF- β can also act by means of non-Smad mediators, such as p38 MAP kinase and Small GTPases, like RhoA. Cytoskeleton and receptor endocytosis are additional mechanisms to regulate the TGF- β signaling. Gene expression can also be eventually modified by micro-RNAs.

Abbreviations: TGF- β , transforming growth factor beta; Smad, second messenger protein; ALKs, activin-like kinases.

In endothelial cells, TGF- β 1 can stimulate ALK-4/5/7 and Smad2/3, or stimulate ALK-1 and Smad1/5/8 and increase cell proliferation.⁸ Smad6 and 7 are inhibitory Smads, since they bind Smad4 and inhibit its internalization into the nucleus.⁹ Endoglin is an accessory to the TGF- β receptor that seems to modulate receptor binding and ALK stimulation.¹⁰ It is mutated in hereditary hemorrhagic telangiectasia type 1,¹¹ whereas ALK-1 is mutated in hereditary hemorrhagic telangiectasia type 2.¹²

In addition to this classical Smad-dependent pathway, other crosstalks have been described among TGF- β 1 and several signaling pathways,¹³ including mitogen-activated protein (MAP) kinases¹⁴ and small GTPases, such as RhoA.¹⁵ Non-Smad pathways seem to be especially important to regulate the TGF- β -mediated fibrotic effect. In particular, focal adhesion kinase has been shown to regulate TGF- β -mediated fibrosis. This is mediated by a TGF- β -mediated recruitment of the p85 subunit of PI3K to focal adhesion kinase to regulate signal transduction, which is independent of tyrosine kinase activation.¹⁶ Among MAP kinases, JNK plays a necessary role in mediating TGF- β -mediated epithelial-to-mesenchymal transition in rat peritoneal fibroblasts, in cooperation with Smad3.¹⁷ In addition, the ERK MAP kinase regulates epithelial-to-mesenchymal transition in mesothelial cells by involving nuclear factor- κ B (NF κ B);¹⁸ Conversely, p38 MAP kinase seems to inhibit this effect in the same cell model.¹⁹ This antifibrotic effect of TGF- β is also mediated by peroxisome proliferator-activated receptor- γ by preventing p300 recruitment, subsequent histone H4 hyperacetylation, and eventual collagen synthesis.²⁰

New insights in TGF- β 1 regulation

In recent years, a growing body of experimental medicine suggests an important role of several factors which may act (in a real-time manner) as rheostats for the fine tuning of the TGF- β 1 pathway, and thus adapt cell response of TGF- β 1 to a given cellular circumstance.

The first important factor is receptor endocytosis. Recent reports indicate that receptor endocytosis is a key event in proper signaling and receptor recycling.²¹ Moreover, it has been clarified that clathrin-coated pits-mediated endocytosis enhances TGF- β function,²² and that early endosomes behave as signaling organelles to promote TGF- β signaling. Conversely, however, the lipid rafts-caveolae endocytic system inhibits TGF- β signaling.²³ Interestingly, it has been described that, given that caveolae localize in cholesterol-rich membrane domains, cholesterol itself inhibits TGF- β signaling in vitro,²⁴ and this might mediate, at least in part, atherogenic effects of cholesterol in vivo. In fact, caveolin, a protein that is a key component of caveolae, physically interacts with TGF- β -R-I to block Smad signaling.²⁵

The second key event is micro-RNA (miRNA) regulation.²⁶ This is achieved by noncoding RNA fragments which are able to silence gene expression. In particular, the miRNA 200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) cooperate with TGF- β signaling to regulate epithelial-to-mesenchymal transition.²⁷ Interestingly, other miRNAs are able to inhibit the antitumor effects of TGF- β and thus are accumulated in human tumors, as seen with the miRNA 25 cluster (miRNA 106b, miRNA 93, and miRNA 25) in gastric cancer,²⁸ neuroblastoma, and multiple myeloma; and the miRNA 17-92 cluster (miRNA 17, miRNA 18a, miRNA 19a, miRNA 20a, miRNA 19b-1, miRNA 92a) in diffuse large B cell lymphoma and small-cell lung cancer.²⁶ MiRNAs miRNA 106b-25 and miR-92 regulate TGF- β -mediated antiproliferative and apoptotic effects.²⁹ In particular, miR-106b and miR-93 inhibit TGF- β -mediated cell cycle arrest, whereas miR-25 inhibits TGF- β -mediated apoptosis.^{26,29} Of note, this crosstalk between the TGF- β pathway and miRNA seems to be a bidirectional process. Thus, miRNA not only affects the TGF- β pathway, but also, miR-21 is directly upregulated by TGF- β 1, and plays a key role in vascular smooth muscle cell differentiation.³⁰ It is expected that this TGF- β -mediated effect also takes place in many other miRNAs. These effects seem to take place postranscriptionally by affecting the Drosha miRNA-stabilizing machinery.²⁶ Therefore, miRNAs are plausible key regulators of the cell response of TGF- β , in a real-time and context-dependent manner.

A third emerging mechanism to control the complex regulation of TGF- β 1 signaling is the cytoskeleton. Smads are tightly anchored to the cytoskeleton and constantly shuttle the cytoplasm and the nucleus in basal cell conditions.³¹ In fact, all Smads are associated to cell microtubules and their trafficking is controlled, at least in part, by microtubules-related proteins, such as kinesin for going to cell membrane receptors,³² and dynein when they direct to the nucleus.³³ In cell culture models, nuclear accumulation of Smads has been considered to be a direct marker of TGF- β 1 signaling,^{34,35} and this could be mediated by nuclear phosphatases, which dephosphorylate the C-terminal di-serine motifs Smads.³⁶

Yet again, this link between TGF- β and cytoskeleton acts in a bidirectional manner, given that TGF- β regulates actin polymerization by the non-Smad-signaling pathways RhoA and p38,³⁷ and by affecting epithelial-to-mesenchymal transition.

In recent years, several mathematical models have been developed that will predict the cell response of TGF- β 1 in a given circumstance *in silico*, and thus help to design target-designed novel molecules, in order to modulate the important roles exerted by TGF- β 1 signaling in health and disease.³⁸ These models are based on a network of molecular components. In order to describe how a given parameter changes with time, systems of ordinary differential equations were incorporated to build a kinetic model, since these could express molecular changes (concentrations and biochemical modifications) over time, and relate these data to empirical ones.³⁸

TGF- β 1 in cardiovascular disease

TGF- β 1 in vascular and hemostasis physiology

In general, TGF- β 1 is considered as an anti-inflammatory cytokine in the vessel wall.³⁹ In normal vessels, TGF- β 1 inhibits endothelial⁴⁰ and vascular smooth muscle⁴¹ cell proliferation. It also increases apoptosis to avoid excessive cellular accumulation,⁴² and stimulates vascular cell differentiation,⁴³ with a parallel decrease of the expression of inflammatory molecules.⁴⁴ In the blood–vessel interface, TGF- β 1 decreases expression of cell adhesion molecules in vascular cells.⁴⁵ In addition, in leukocytes, it decreases the activation of integrins⁴⁶ and stimulates the function of endothelial progenitor cells, which may help to restore the denuded vessel wall.⁴⁷ In hemostasis, TGF- β 1 seems to behave as an antifibrinolytic factor⁴⁸ and stimulates platelet-induced vascular repair.⁴⁹ TGF- β 1 is a normal component of platelet alpha granules.⁵⁰ In fact, the vast majority of serum TGF- β 1 comes from platelet degranulation.⁵¹

TGF- β 1 in atherosclerosis

In atherosclerosis, TGF- β 1 is considered to be an antiatherogenic factor, especially in the early stages of the disease, according to what has been termed the protective cytokine hypothesis.⁵² Thus, TGF- β 1 inhibits excessive vascular smooth muscle cell accumulation in the neointima,⁵³ and avoids plaque rupture by means of its stimulation of extracellular matrix synthesis and tissue repair.⁵⁴ In addition, it controls local inflammation by stimulating Th3 function and regulatory T cells (CD3⁺/CD25⁺ cells).⁵⁵ Therefore, it avoids the excessive immune attack (from both innate and acquired immunity mechanisms) that characterizes atherosclerotic vascular disease.⁵⁶ In the clinical arena, decreased serum levels of TGF- β 1 have been correlated to clinical atherosclerosis.⁵¹ However, this parameter has remained an elusive atherosclerosis marker, given that these levels may vary according to the time course of the disease and age.⁵⁷ The majority of serum TGF- β levels originate from platelets and thus are markers of platelet activation in atherosclerosis² and rheumatoid arthritis.⁵⁸ TGF- β levels can be calculated as an active or total (active and acid-activatable) form. In systemic lupus erythematosus patients, a lower serum activation index has been associated with increased lymphocyte apoptosis, irreversible organ damage, disease duration, low-density lipoprotein, and increased carotid intima-media thickness.⁵⁹ Endoglin is an accessory TGF- β receptor and soluble endoglin may interfere with TGF- β interaction with membrane-bound receptors and thus decrease TGF- β signaling.⁶⁰ Increased levels of soluble endoglin have been related to atherosclerosis,⁶¹ as well as preeclampsia.⁶²

However, in late stages of the disease, TGF- β 1 seems to behave as a proatherogenic factor by increasing excessive extracellular matrix,⁶³ promotion of in-stent restenosis,⁶⁴ and induction of pathologic vascular remodeling.⁶⁵ In fact, it has been demonstrated that end-organ damage in hypertension has been related to increased levels of TGF- β 1 in serum and urine.⁶⁶ Moreover, in atherosclerotic vascular disease, it has been demonstrated that cells become insensitive to TGF- β 1 signaling by means of decreased TGF- β 1 activation,⁶⁷ decreased receptor^{2,68} and Smad downregulation,^{2,69} altered endocytosis and intracellular trafficking pattern,²¹ or alteration of any of the other multiple cellular pathways that crosstalk with the TGF- β 1 signaling pathway.² Interestingly, many of these factors may be altered at the genetic level by means of congenital atherosclerotic-related polymorphisms^{70–72} and genetic determinants of aortic aneurysms.⁷³ Moreover, even acquired mutations have been postulated to modulate these facts.⁷⁴

TGF- β 1 in myeloproliferative syndromes

Philadelphia-negative myeloproliferative syndromes (polycythemia vera, essential thrombocythosis, essential myelofibrosis) are clonal hematological neoplasms in which an increased risk of arterial thrombosis occurs. A growing body of clinical and experimental evidence suggests that these variable phenotypes can follow a graded natural history, from initial essential thrombocythosis to subsequent polycythemia vera, and eventual spent-phase secondary myelofibrosis.⁷⁵ These three disorders are associated with increased risk of arterial thrombosis, and in polycythemia vera, this risk is 20% at 10 years.⁷⁵

In essential thrombocythosis, the abnormal clone seems to lose sensitivity to the proliferation-control effects of TGF- β 1.⁷⁶ In polycythemia vera, the abnormal clone equally loses response to the cytokine.⁷⁷

However, essential or secondary myelofibrosis is the chronic myeloproliferative syndrome which is associated with the shortest survival rate.⁷⁸ The risk of thrombosis is similar to that found in essential thrombocythemia (1%–3% per patient per year).⁷⁹ This is the variant of myeloproliferative syndrome which has the strongest link to dysregulation of the TGF- β 1 pathway.⁷⁸ In fact, TGF- β increases myelofibrosis in murine models.⁸⁰ In cell culture models, TGF- β secretion seems to be regulated by NF κ B.⁸¹ In myeloproliferative syndromes, there seems to be a progressive grading in TGF- β 1 in the prefibrotic state, although established myelofibrosis has lower TGF- β 1 levels.⁸² Higher TGF- β levels seem to be correlated with increased allelic charge of JAK2, and decreased EPC levels.^{82,83}

Therapy of myelofibrosis is a clinical challenge and supportive care has been the only treatment to date.⁷⁷ Current JAK-2 inhibitors have shown only a limited benefit in regard to spleen size.⁸⁴ Advances in the knowledge of TGF- β signaling in myeloproliferative syndromes may guide the choice of synergistic novel therapies such as small-molecule TGF- β pathway blockers including, SB-431542/ALK-4 inhibitor of the ALK-4 kinase activity², heat-shock protein inhibitors, NF κ B inhibitors,⁸⁵ or epigenetic drugs.⁸⁶

Thus, progressive TGF- β dysfunction can be considered as a shared pathogenic event in atherosclerosis and Philadelphia-negative myeloproliferative syndromes, and a putative diagnostic and therapeutic tool.

Conclusion

TGF- β 1 is a key factor in diseases affected by cardiovascular disorders, such as atherosclerosis and myeloproliferative

syndromes. The current knowledge of the complex TGF- β 1 regulation of physiological and pathological processes may help to design novel diagnostic techniques and target-designed innovative therapies.

Authors' contributions

SR conceived the idea of the manuscript and wrote the first schematic draft. SR and TT designed the search strategy. SR, JN-D, MR, UM, and TT performed the review of the literature. All authors collaborated in the definitive version, and approved the final draft of the manuscript.

Acknowledgment/disclosure

Our lab received support by FISS (Health Research Fund, PI080920) and Red Temática de Investigación Cardiovascular RECAVA (RD06/0014/1007), both from the Instituto de Salud Carlos III, Spanish Ministry of Health (ISCIII). The authors report no conflicts of interest in this work.

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