Transforming Growth Factor-β (TGF-β) is the prototype of a superfamily of multifunctional proteins which includes activins and bone morphogenetic proteins (BMPs). TGF-β is involved in the regulation of diverse cellular processes such as cell proliferation, differentiation, apoptosis and migration, as well as moderating cell–cell and cell–matrix interactions. It plays a pivotal role in embryonic development, and perturbations of TGF-β cell–matrix interactions. It plays a pivotal role in embryonic development, and perturbations of TGF-β signaling may have profound pathological consequences in a wide variety of diseases. (For general reviews on TGF-β actions, please see Massagué1 and Kim et al.2)

Cellular responses to TGF-β are complex and, depending on cell type and conditions, it can induce stimulatory or inhibitory effects. In the heart, TGF-β stimulates hypertrophy of cardiomyocytes and proliferation of fibroblasts, contributing to myocardial fibrosis and dysfunction.3 In the blood vessel wall, TGF-β inhibits proliferation and migration of vascular smooth muscle cells, and defective signaling can lead to pathological vascular remodeling.4 TGF-β is also a potent suppressor of leukocyte activation, and under some conditions can exert a protective immunomodulatory role in atherosclerosis.5,6 TGF-β can act as an inhibitor or a stimulator of endothelial proliferation, impacting on different stages of angiogenesis.7,8 Despite the diversity in actions, a common mediator of TGF-β signaling is involved in transcriptional regulation, MH1 can also bind to the TGF-β gene.9

Because TGF-β is involved in such a vast diversity of cellular events, it is therefore not surprising that its signaling is characterized by a remarkable degree of complexity and subtlety. Members of the TGF-β superfamily interact with 2 classes of serine/threonine receptors. TGF-β binds avidly to its type II receptor (TβR-II), and the receptor–ligand complex then recruits TβR-I (ALK5). The incorporation of the type I and II receptors into a close spatial arrangement leads to activation of these receptor complexes resulting from the phosphorylation of the type I receptor by the constitutively active type II receptor. Receptor activation leads to the phosphorylation of a family of cytoplasmic proteins which were initially identified in Drosophila and termed mothers against decapentaplegic (Mad), the vertebrate homologues of which are the so-called Smads.10 Eight Smad members are known in mammals, classified into 3 different groups based on structure and function: receptor-regulated Smads (R-Smad), which include Smads 1, 2, 3, 5, and 8; a common-mediator Smad (co-Smad), Smad4; and Inhibitory (I) Smads 6 and 7. The signaling from the TβRII/RII complex is mediated by Smads 2 and 3. In endothelial cells, an alternate receptor–ligand complex can be formed with ALK1 as the type I receptor, which employs Smads 1 and 5. A balance between the two signaling pathways, which lead to the activation of different sets of genes, may constitute an on/off switch for the control of angiogenesis.8 In both cases, phosphorylation of the R-Smad results in increased association with Smad4 (Co-Smad) and translocation of the complex to the nucleus. Another level of complexity is introduced by the existence of two functional Smad domains, MH1 and MH2, which interact, often in opposite ways, to regulate gene transcription. Although both domains can interact with a wide variety of nuclear proteins involved in transcriptional regulation, MH1 can also bind to Smad-binding element of the DNA directly. The existence of different transcription factors and cofactors in various cell types has been shown to account for some of the cell-specificity of TGF-β responses.10

There are several intricate layers of regulation of TGF-β signaling which include inhibition of the extracellular receptor binding of TGF-β (and other members of this superfamily) by soluble proteins (ie, decorin, α2-macroglobulin, and Noggin), the presence of membrane anchored accessory (ie, betaglycan, endoglin) or decoy receptors (ie, BAMBI), as well as intracellular proteins, such as FKBP12 which binds to the unphosphorylated GS domain of type I receptors thus reducing basal activity. A further level of complexity is provided by the mechanisms of inactivation of Smad signaling, which involve both additional phosphorylation and dephosphorylation by as yet poorly understood phosphatases, as well as ubiquination. A diverse range of cytoplasmic kinases have been shown to phosphorylate the linker regions of R-Smads, inhibiting ligand-induced nuclear translocation.10,11 Ubiquination involves a family of E3 ubiquitin ligases, the Smad ubiquination regulatory factors (Smurfs) which target the activated R-Smads and TGF-β receptors to the proteasome for degradation. Again the precise mechanisms vary for the signaling pathways of various members of the TGF-β superfamily: Smurf1 targets Smads 1 and 5 for destruction in the cytoplasm of unstimulated cells, whereas activated Smad2 is ubiquinated in the nucleus by a Smurf2-dependent mechanism.12
Into this complex fabric of regulation, Saura et al now weave an additional thread that may have important implications regarding context and cell-dependent effects of TGF-β signaling. In this issue of Circulation Research, they report that nitric oxide (NO) inhibited TGF-β autoinduction and signaling in endothelial cells by enhancing proteasomal degradation of the activated Smad2/3. They suggest that this inhibition may serve as a molecular restraint thus preventing excessive actions of this pathway, as has been implicated in maladaptive cardiac and vascular remodeling in pathological conditions such as heart failure and atherosclerosis. Central to this concept is the fact that TGF-β induces the expression of both eNOSs and TGF-β genes via Smad2 in endothelial cells. Increased NO production and cGMP-dependent protein kinase (PKG) activity lead to the ubiquination and targeted destruction of phosphorylated Smad2 by the proteasome. As the level of activated Smad2 diminishes, Smad2-mediated induction of TGF-β (and eNOS) would then cease, completing the feedback loop. Under this proposed scheme, a lack of NO production, for instance, in endothelial dysfunction, would interfere with this protective restraint, leading to unfeathered autoinduction of TGF-β and potential deleterious effects of excessive TGF-β production on vascular remodeling and fibrosis.

A limitation of the present study is that the relevance of this paradigm was not tested directly in vivo, for example in models of atherosclerosis. The role of TGF-β in atherosclerosis is complex, and rather than contributing to the pathogenesis of this disease, there is a body of evidence that supports an atheroprotective role, both by inhibiting smooth muscle cell proliferation and maintaining vascular wall homeostasis and also by antiinflammatory actions, reducing T-cell and macrophage activation. Thus, rather than being beneficial, suppression of TGF-β signaling may in fact be detrimental in atherosclerotic models. Also, one cannot exclude that in advanced atherosclerotic plaque, the reduced expression of matrix genes as described in this report may have another deleterious effect, weakening the fibrous cap and thus contributing to plaque instability.

There are a number of other questions that remain to be addressed regarding NO and TGF-β signaling. Although Smad2-dependent matrix deposition may be inhibited by TGF-β-induced NO production, signaling pathways mediated by other Smads could still be active. Smad3, whose fate was not clearly defined by Saura et al, has been shown to mediate TGF-β-induced expression of Endothelin-1 and platelet-derived growth factor. As well, the mechanism by which NO enhances degradation of Smad2 was not well defined. Although no change was seen in overall expression of Smad7, one cannot exclude a role for this I-Smad which is known to participate in targeting Smad2 for ubiquination and proteasome-mediated degradation. Smad7 normally resides in the nucleus under basal conditions and translocates to the plasma membrane on TGF-β stimulation, playing a critical role in the regulation of Smurf-mediated ubiquination of the receptor complex and its subsequent degradation. Both Smurf1 and Smurf2 bind to Smad7, and this complex in turn interacts with the activated type I receptor, inhibiting R-Smad phosphorylation as well as mediating receptor ubiquination and degradation of the receptor–Smad7 complex. At the same time, steady-state levels of Smad7 are maintained by the transcriptional activation of Smad7 by TGF-β. Although Saura et al provide convincing data in support of a cGMP-PKG–mediated mechanism, they pointedly leave the door open in the discussion by suggesting that it may also reduce Smad binding to DNA by S-nitrosylation. Finally, one may also question how these data relate to the recent observation by Toporsian et al that endoglin, an accessory receptor to the TGF-β receptor complex in endothelial cells, serves to couple the activation of this receptor to NO.

Could perhaps this mechanism also contribute to differences in response to TGF-β in endothelial cells compared with other cell types that do not normally elaborate NO? TGF-β has cell type–specific effects on the fate of vascular cells; similar doses of TGF-β are proapoptotic to endothelial cells and antia apoptotic to vascular smooth muscle cells, and these effects can be modulated by cell–matrix interactions. Also, it would be of interest to know to what extent endothelial-derived NO production might contribute to the recently described differential effects of BMPs on growth and survival of endothelial and smooth muscle cells, which may be critical in the pathogenesis of pulmonary arterial hypertension. As noted by the authors, whereas NO prevents TGF-β induction in endothelial cells, it can have an opposite effect in smooth muscle cells, and exogenous NO has been shown to result in the overexpression of TGF-β, leading to excessive production of extracellular matrix. Thus, NO may modulate TGF-β signaling pathways quite differently in a cell-type specific manner; the challenge remains to decipher the implications of intricate TGF-β modulatory mechanisms, such as NO, on the regulation of pathophysiological events in the cardiovascular system.

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References


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