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Review article

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TGF-β SIGNALING PATHWAY: ITS ROLE IN GASTROINTESTINAL PATHOPHYSIOLOGY AND MODULATION OF ULCER HEALING

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Gastrointestinal ulcer healing is a complex process, involving cell migration, proliferation, angiogenesis and extracellular matrix deposition, all ultimately leading to reconstruction of tissue architecture within the ulcer scar. These processes are controlled by growth factors, cytokines and hormones. Transforming growth factor-β (TGF-β), one of the multifunctional peptide growth factors, has been reported to positively regulate gastrointestinal ulcer healing. Although TGF-β inhibits cell proliferation in a variety of cells, it induces cell migration, angiogenesis, and enhances extracellular matrix production necessary for gastrointestinal ulcer healing. TGF-β exerts its action by binding to its transmembrane serine/threonine kinase receptors, which in turn triggers activation of various intracellular signaling pathways. Smads are intermediate effector proteins that play key roles in biological activities of TGF-β by transmitting the signals from the cell surface directly into the nucleus and initiating transcription. New insight into the mechanisms underlying TGF-β-Smad modulation of gastrointestinal ulcer healing will likely enhance our understanding of the mechanisms controlling the healing processes of gastrointestinal ulcers.

Key words: *TGF-β, smad, gastrointestinal ulcer healing*

INTRODUCTION

Ulcer in gastrointestinal tract develops as a result of imbalance between mucosal defensive (protective) factors such as mucosal blood flow, ischemic preconditioning, nitric oxide and prostaglandin generation, growth factors, ghrelin and others, and aggressive factors such as HCl, pepsin, bile acids and

others (1-4). Ulcer healing is a complex process, involving cell migration, proliferation, angiogenesis and extracellular matrix deposition, all ultimately leading to scar formation (5-8). All these processes are controlled by growth factors such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), basic fibroblast growth factor (bFGF), trefoil peptides (TP), platelet-derived growth factor (PDGF) and a variety of cytokines (6-9).

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that regulates many diverse cellular processes including proliferation, apoptosis and differentiation. TGF-β is known to play a central role in different stages of wound healing (10). TGF-β is also reported to positively regulate gastrointestinal ulcer healing, but little is known about the precise role of TGF-β in healing process. This review summarizes recent advances in the understanding of the mechanisms underlying TGF-β modulation of gastrointestinal ulcer healing.

TGF-β

TGF-β is a 25-kDa disulfide-linked homodimeric peptide. Three mammalian isoforms (TGF-β1 -β2 and -β3) have been identified that share a 64-85% amino acid sequence homology. TGF-β1 is the prevalent form and found almost ubiquitously (reviewed in 11, 12). Other isoforms are expressed in a more limited variety of cells and tissues. The gene encoding TGF-β1, -β2 and -β3 are located in 19q13, 1q41 and 14q24, respectively. While TGF-β stimulates proliferation of fibroblasts (13), it inhibits cell proliferation in a variety of cells (e.g. epithelial cells and endothelial cells).

TGF-β is synthesized as a large precursor that is subsequently cleaved. The cleaved pro-region known as the latency-associated peptide (LAP) has been shown to remain non-covalently associated with the mature peptide to form a latent TGF-β1 complex, also known as the small latent complex (SLC). SLC is secreted and undergoes further processing in the extracellular matrix, which involves proteases such as plasmin, integrin-αvβ6, mannose-6-phosphate receptors, plasmin, matrix metalloproteinase-2 and -9, thrombospondin-1, and cathepsin D.

TGF-β SIGNALING PATHWAY

TGF-β elicits its cellular response by binding to heteromeric complex of type I and type II serine/threonine kinase transmembrane receptors, which in turn activates multiple downstream signaling pathways resulting in several distinct effects (*Figure 1*). The first intracellular mediator of TGF-β signaling, Smad [ortholog of mothers against dpp (MAD) first identified in Drosophila] (14), transduces the TGF-β signal from the plasma membrane to the nucleus and play key roles in biological activities of TGF-β. Among the three classes of Smads, only

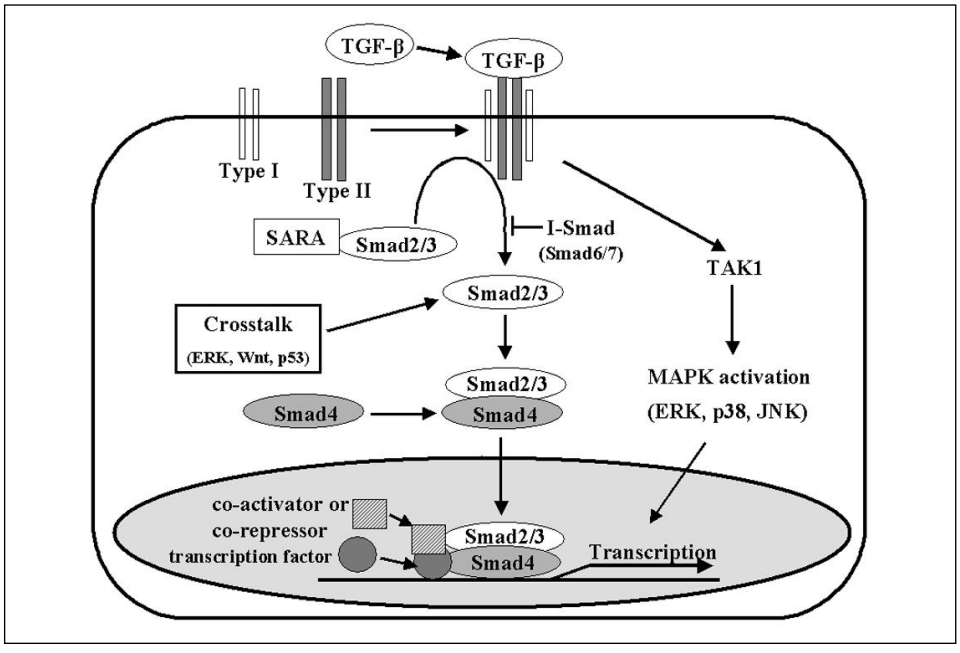

Fig.1. TGF-β signaling pathways. Classically, TGF-β binds to type II receptor on cell membrane, which induces the formation of type I receptor and type II receptor complex. Type II receptor phosphorylates the GS domain of type I receptor. The activated type I receptor then specifically phosphorylates Smad2/3. Phosphorylated Smad2/3 form complexes with Smad4. This Smad complex translocates into the nucleus and participates in transcriptional regulation. Recently, it has been shown that cross-talk between other pathways modulate TGF-β-Smad signaling pathway, which include MAPK, Wnt and p53 pathways.

receptor-regulated Smads (R-Smad) are directly phosphorylated and activated by type I receptor kinases. Among the multiple intracellular signaling pathways activated, Smad signaling pathway is relatively well elucidated (reviewed in 11, 12). Type III receptors containing a proteoglycan and a glycoprotein known as betaglycan and endoglin, likely modulate activity by regulating ligand access to type I and type II receptors, without transducing signal by itself.

Type I and type II receptors are known to act in a sequence to exert the TGF-β signal. First, TGF-β binds to type II receptor, which in turn recruits the cognate type I receptor into the complex. Once the complex is formed, type II receptor kinase phosphorylates glycine- and serine-rich juxtamembrane regions within type I receptor called the GS domain, resulting in induction of kinase activity of type I receptor. The activated type I receptor then specifically recognizes and phosphorylates R-Smad, which include Smad2 and Smad3. In the basal state, R-Smads can bind several proteins including SARA (Smad anchor for receptor activation) (*Figure 1*). SARA presents R-Smads as substrates to the activated TGF-β receptor complex and their phosphorylation decreases the affinity of R-

Smads for SARA. Once released from SARA, phosphorylated Smad2/3 form complexes with Smad4, which is called co-mediator Smad (Co-Smad). This Smads complex translocates into the nucleus and participates in transcriptional regulation. Smad3 and Smad4 bind directly to DNA via a GC-rich consensus sequence called Smad-binding element. In most cases of transcriptional activation, Smad requires binding of additional transcription factors such as activator protein (AP-1), Fast-1, Fast-2, transcription factor muE3 (TFE3), simian virus 40 promoter factor 1(Sp-1). Activated Smads in the cell nucleus can also interact with transcriptional co-activators including cAMP response element binding protein (CBP) and p300, or with co-repressors including TG-interacting factor (TGIF), c-Ski, and SnoN, for both activation and inhibition of transcription.

Smad signaling pathway is essential for most TGF-β responses. Recently it has been shown that cross-talk between other signaling pathways modulate TGF-β-Smad signaling pathway. TGF-β has been shown to activate extracellular-signal-regulated kinase (ERK)-1, ERK-2, p38 or c-Jun amino-terminal kinase (JNK) also known as mitogen-activated protein kinases (MAPKs) (15-17). These Smad-independent TGF-β signaling pathways are mediated in part by TAK1, a MAPK kinase kinase (18). Furthermore, TGF-β and Wnt pathway effectors have been shown to interact directly (19). The Wnt (ortholog of segment polarity gene 'wingless' in Drosophila) genes encode a large family of secreted protein growth factors that have been identified in a wide variety of animals including hydra and humans. During development, Wnts are known to govern cell fate, proliferation, migration, polarity and death during development, while in adults, Wnts are known to function in homeostasis, and aberrant activation of Wnt pathway is implicated in various types of cancers (20). TGF-β-dependent interaction between Smad3 and transcription factor-lymphocyte enhancer factor 1 (Lef1) has been shown to regulate synergistic induction of Wnt target genes (21). Interestingly, the inhibitory Wnt pathway protein, axin has been shown to associate with Smad3 and facilitate its phosphorylation by TGF-β receptors (22). A recent study demonstrated that members of p53 family act synergistically with the Smad complex to control gene expression, and that several TGF-β target genes were under control of p53 and Smad in mammalian cells (23).

EXPRESSION OF TGF-β IN GASTROINTESTINAL TRACT

While varied expression and localization of all three isoforms of TGF-β have been demonstrated in both the adult and the embryonic intestine, localization of TGF-β and its receptors in gastrointestinal mucosa remains controversial (24-35) (*Table 1*). An earlier study reported that all three isoforms of TGF-β colocalize predominantly to the villus tip of intestine and colon (25). Dunker and co-workers indicated that TGF-β2 is detected in endocrine cells and TGF-β3 is predominantly found in goblet cells (30). Immunoreactive TGF-β1 protein has been detected in human gastric mucosa (31, 32). Clear expressions of TGF-β1, -

Table 1. The localization of TGF-β in gastrointestinal tract.

organ	localization	isoforms	reference
Intestine	mucosal epithelium	TGF-β1	24
	villus tip cells of the epithelium	TGF-β1, 2, and 3	25, 26
	submucosal region just basal to the epithelium	TGF-β2	26
	crypt cells	TGF-β1	28
	endocrine cells	TGF-β2	30
	goblet cells	TGF-β3	30
Stomach	submucosal region just basal to the epithelium	TGF-β2	26
	chief cells and parietal cells of the fundic gland	TGF-β1, 2 and 3	32
	parietal cells, mucus cells	TGF-β1	33
	chief cells	TGF-β2	33
	parietal cells, mucus cells, chief cells	TGF-β3	33
	epithelial cells beneath the proliferative zone	TGF-β1	35

β2, -β3, type I and type II receptors in chief and parietal cells of the fundic glands have been documented (32). In contrast, Naef and co-workers showed that TGF-β1 is localized mainly to parietal cells and also to some surface mucus cells, TGF-β2 was present exclusively in chief cells and TGF-β3 was present in parietal, chief and mucus cells (33). Another study reported that gastric fibroblasts of human stomach occasionally showed immunoreactivity for proTGF-β1 (TGF-β1 precursor), whereas epithelial cells were all negative (34). In rat stomach, TGF-β mRNA in normal gastric mucosa (35, 36) and immunoreactive TGF-β1 protein exclusively localized to the epithelial cells beneath the proliferative zone in the gastric glands has been reported (35).

The precise role of constitutive TGF-β in normal gastrointestinal tract remains unknown. Several reports suggest that TGF-β1 may function as a regulator of epithelial morphogenesis in the gastrointestinal tract. In TGF-β1 heterozygous mice, hyperplastic lesions similar to human gastritis cystica profunda were observed (37). An earlier study indicated that laminins and TGF-β maintain cell polarity and function of human gastric glandular epithelium and regulate the architecture of gastric glands (38). In TGF-β2 and -β3 heterozygous mice, programmed cell death was significantly reduced in the intestinal mucosa accompanied by an increase in villus length and upregulation of Bcl-xL and Bcl-2 (30), suggesting that TGF-β may play an important role in the control of growth and differentiation in the gastrointestinal mucosa and may function in gastrointestinal epithelium as a coordinator of cell turnover.

TGF-β AND GASTROINTESTINAL ULCER HEALING

Recent studies showed that TGF-β accelerates healing of experimental dermal ulcer and incisional dermal wounds (39-41) by regulating migration, proliferation, and differentiation of various cells and by stimulating synthesis of extracellular matrix and angiogenesis (10, 42, 43).

Similarly, it has been suggested that TGF-β accelerates gastrointestinal ulcer healing by regulating crucial processes such as proliferation and migration of epithelial cells and fibroblasts, formation of granulation tissue, deposition of extracellular matrix and promoting angiogenesis. In experimental gastric ulcer, TGF-β is overexpressed in the granulation tissue (35, 36). TGF-β1 is mainly derived from macrophages, polymorphonuclear cells, fibroblasts and myofibroblasts in the granulation tissues of gastric ulcer (35). Exogenous TGF-β accelerates gastrointestinal mucosal wound repair *in vitro* (44-46) and *in vivo* (41, 47, 48). While local and systemic TGF-β3 treatment has been shown to accelerate gastric ulcer healing in rats (47), Ernst and co-workers reported that injection of TGF-β1 into the subserosa around the experimental gastric ulcer accelerate gastric ulcer healing (48). Blocking of TGF-β type II receptor using dominant-negative type II receptor constructs results in impairment of mucosal healing in dextran sulfate sodium-induced colitis model and *in vitro* wound-healing model (49). Clinical studies show that patients with healed gastric ulcers showed increased expression levels of both TGF-β and its receptors while patients with refractory ulcers had weak or deficient TGF-β expression in the gastric mucosa, suggesting crucial role of TGF-β in gastric ulcer healing (50).

Clinical studies demonstrate that prostaglandins of E series (PGEs) and their synthetic analogs facilitate healing of gastroduodenal ulcers, dermal ulcers and wounds (51-54). In addition, a previous study also demonstrated that treatment with a synthetic PGE1 analog with agonistic activity to EP1-4 receptors, misoprostol accelerated non-steroidal anti-inflammatory drugs-induced gastric ulcers (52). Experimental studies in rats demonstrated that treatment with PGE1 stimulated TGF-β1 expression and this increase was associated with the accelerated gastric ulcer healing. Conversely, treatment with combination of PGE1 and indomethacin, which inhibits prostaglandin synthesis, reduced TGF-β1 expression and delayed ulcer healing. These studies indicate important role of TGF-β1 in PGE1-promoted gastric ulcer healing (35).

TGF-β has also been reported to regulate the cyclooxygenase-2 (COX-2) expression. Takahashi and co-workers demonstrated *in in vivo* and *in vitro* studies that COX-2 protein is localized to the base of gastric ulcers in rats and that COX-2 mRNA expression is regulated positively by IL-1β and TNF-α and negatively by TGF-β1 (36). Thus, TGF-β1 plays important roles in the process of gastric ulcer healing where it interacts with prostaglandins and COX-2.

MECHANISMS OF TGF-β PROMOTED GASTROINTESTINAL ULCER HEALING

The effect of TGF-β on cell proliferation and migration of epithelial cells

Re-epithelialization is an essential process for cutaneous and gastrointestinal wound/ulcer healing (6, 8). TGF-β accelerates mucosal re-epithelialization by promoting cell migration. The addition of TGF-β1 to wounded rat intestinal

epithelial cell monolayers inhibits proliferation, but stimulates migration of these cells and subsequently promotes restitution (44). Inactivation of TGF-β1 by using specific neutralizing antibody inhibited the restitution promoted by EGF, TGF-α, Interleukin-1β and Interferon-γ (45). Blockade of intestinal epithelial TGF-β activity by dominant-negative TGF-β type II receptor impaired intestinal wound healing *in vitro* and *in vivo* (49). Similarly, it has been shown that gastric mucosal cell monolayers restitution was retarded by neutralization of endogenous TGF-β with anti-TGF-β antibody while were restored by human recombinant TGF-β treatment. This suggests that TGF-β is required for re-epithelialization of gastric ulcer during healing (46).

Numerous mechanisms by which TGF-β might stimulate cell migration have been proposed. It has been suggested that TGF-β strengthens attachment of migrating epithelial cells to basement membrane collagens by regulating expression in the epithelial cells of extracellular matrix receptor such as integrin, fibronectin, vitronectin and laminin, which facilitate keratinocyte migration (55-57). It has been also suggested that TGF-β may directly stimulate cell motility by modulating a hyaluronan receptor and reorganizing actin cytoskeleton (58, 59).

TGF-β and angiogenesis during gastrointestinal ulcer healing

Angiogenesis - formation of a new microvascular network - is a major component of wound healing and tissue regeneration. It is important for the repair of acute gastric mucosal injury and for chronic gastrointestinal ulcer healing (6-9). Numerous studies have demonstrated that the growth of granulation tissue and generation of new microvessels through angiogenesis is stimulated by basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and angiopoietins and possibly by other growth factors and cytokines, including interleukin-1 and tumor necrosis factor-α (60, 61).

Recent studies suggest that TGF-β can also trigger angiogenesis by inducing expression of angiogenic factors such as VEGF in both epithelial cells and fibroblasts (62, 63). In addition, TGF-β induces the expression and secretion of matrix metalloproteinases leading to capillary basement membrane dissolution allowing endothelial cells migration, essential for angiogenesis (64).

Induction of extracellular matrix by TGF-β

Extracellular matrix such as collagen types I, III, IV, fibronectin and laminin in the interstitium and in the basement membrane has been implicated in cell adhesion, migration, and proliferation during gastric ulcer healing (35, 65, 66). In addition, TGF-β is implicated in the formation of granulation tissues and the re-establishment of basement membrane allowing re-epithelialization. TGF-β is a potent stimulator of the expression of extracellular matrix proteins during ulcer healing (11).

PERSPECTIVES

While a significant progress in understanding the cellular and molecular mechanisms of gastrointestinal ulcer healing and involvement of TGF-β in this process has been recently accomplished, the precise roles of TGF-β in healing of gastrointestinal ulcers remain unclear. This is partly due to cell-type specific effect of TGF-β and cross-talk with other signaling pathways. For example, in contrast to predictions made on the basis of the ability of exogenous TGF-β to improve wound healing, Smad3-null ($Smad3^{ex8/ex8}$) mice paradoxically show accelerated cutaneous wound healing compared to wild-type mice, reflected by increased re-epithelialization and significantly reduced local monocytic infiltration (67). Similarly, re-epithelialization of large intestine after injury by 2, 4, 6-trinitrobenzene sulfonic acid is faster in Smad3 heterozygous mice than in wild-type littermates even if there is no difference in the degree of mucosal inflammation between the two groups (68). However, overexpression of Smad3 targeting mainly dermal fibroblasts by subcutaneous injection of adenovirus containing Smad3 complementary DNA accelerates wound healing with upregulation of α-smooth muscle actin, VEGF and fibroblast growth factor receptor (69). Further elucidation of cell type-specific roles of TGF-β-Smad signaling pathway and the downstream effects of various cross-talks would enhance our understanding of the mechanisms controlling the healing processes of gastrointestinal ulcers and could help develop new therapeutic modalities.

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