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Review Article

Regulation and Signaling of TGF-β Autoinduction

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Cell signaling is a vital part of biological life. It helps coordinating various cellular processes including cell survival, cell growth, cell death, and cell interaction with the microenvironment and other cells. In general, cell signaling involves the attachment of signaling molecules known as ligands to specific receptors on cell surface, which then activate downstream events that dictate the cell's response. One of the most studied ligands is transforming growth factor-beta (TGF-β). TGF-β signaling is mainly mediated by suppressor of mothers against decapentaplegic (Smad) proteins, but it also interacts with other pathways such as the Ras and mitogen-activated protein kinase (MAPK) signaling pathways. Furthermore, TGF-β can have a dual role depending on the cellular and microenvironmental context, in which it can act as either a growth promoter or a growth inhibitor. It has been known that TGF-β can self-induce its ligand production, thereby prolonging and amplifying its effect on cells and their microenvironment. The aim of this review is to discuss the regulation and signaling of TGF-β autoinduction, which still remain to be elucidated. Several factors have been found to facilitate TGF-β autoinduction, which include the activator protein-1 (AP1) complex, Smad3-dependent signaling, and non-Smad signaling pathways. On the other hand, the LIM (Lin11, Isl-1 and Mec-3) domain only 7 (LMO7) protein can suppress TGF-β autoinduction by interfering with the activities of AP-1 and Smad3. Since TGF-β autoinduction is implicated in various pathological conditions, better understanding of its regulation and signaling can provide new directions for therapy.

Key words: Cell signaling, TGF-β, TGF-β receptors, autoinduction, signaling pathways, Smad-dependent signaling, non-Smad-dependent signaling

The information exchange between cells and their microenvironment is crucial for cell survival. The message conveyed can be related to the extracellular physical and chemical changes as well as communication signals from other cells. This phenomenon is generally known as cell signaling, which then triggers intracellular network modulation that enables cells to deliver an appropriate response (1). Most cellular signaling molecules, called ligands, will be captured and

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bound to their specific receptors. Their binding then triggers a series of biochemical reactions that promote cellular responses such as proliferation, differentiation, and specific metabolic reactions. Ligands can exist in the form of hormones, cytokines, chemokines, and growth factors (1).

Among many growth factors, the transforming growth factor-beta (TGF-β) attracts great interest due to its pivotal role in the regulation of cell cycle, growth, and development. Moreover, TGF-β signaling is also involved in the pathogenesis of various diseases like cancer (2-4). Interestingly, the TGF-β ligand is able to self-regulate and self-induce its production via a signaling pathway known as autoinduction (5). This ability can augment its role in both physiological and pathological processes. In particular, the clinical implications of TGF-β autoinduction on cancer are worthy to be explored in order to provide new directions for therapy. This review will discuss the regulation of TGF-β signaling, its autoinduction mechanism, and the role of TGF-β autoinduction in pathophysiological processes particularly cancer.

TGF-β family and isoforms

The molecular cloning of TGF-β complementary DNA (cDNA) that began in 1985 has made it possible to identify TGF-β family and superfamily members and their structural biology (6). TGF-β superfamily comprises a large group of regulatory proteins which share common biological roles and structural homology. It consists of several families, including TGF-β, bone morphogenetic proteins (BMPs), growth and differentiation factors, activins and inhibins, mullerian inhibiting substance, left-right determination factor, and nodal growth differentiation factor (7). Despite the diversity, all superfamily members have comparable structural links and exhibit similar synthesis schemes. They start from a much larger precursor polypeptide. Precursor polypeptides consist of three segments: the peptide segment of the amino-terminal hydrophobic signal that will be released when the protein translocated into the lumen of the rough endoplasmic reticulum, the precursor segment or the N-terminal pro-domain, and the C-terminal segment that will become an active mature peptide (7-9).

The TGF-β family members are multifunctional proteins involved in numerous cellular processes like tissue repair and remodelling (10-12). In addition, by exhibiting immunos-uppressive and anti-inflammatory activities, they also regulate the immune system (13). There are three well-known TGF-β isoforms, TGF-β1, TGF-β2, TGF-β3, that are found in mammals and share similarity in their amino acid sequence (9, 14).

Three different genes on different chromosomes are responsible for coding the TGF-β isoforms in humans. Respectively, *TGF-β1*, *TGF-β2*, and *TGF-β3* genes are located on the 19q13, 1q41, and 14q24 of human chromosomes (Fig. 1). They are expressed in a controlled manner, adapting to specific stages of tissue development. *TGF-β1* is expressed in endothelial cells, hematopoietic, and connective tissue, while *TGF-β2* is expressed in epithelial cells and nerve cells, and *TGF-β3* gene mainly in mesenchymal cells (14). Moreover, each TGF-β isoform also has a different affinity for TGF-β receptors. Promoter segments-wise, *TGF-β1* is the only one that has no classic TATAA box. Instead, it incorporates several regulatory sites such as activator protein-1 (AP-1), specificity protein-1 (Sp-1), early growth response factor-1 (EGR-1), and Ras converting CAAX endopeptidase (RCE) as stated in GeneCards (GC19M041301) (Fig. 2A). In contrast, *TGF-β2* and *TGF-β3* promoters contain a typical TATAA box and a proximal ATF/cAMP response element (ATF/CRE) section (GC01P218345 and GC14M075958, respectively) (Fig. 2B, C). Moreover, the *TGF-β3* promoter comprises a repetitive TCCC motif in the upstream part that is crucial for high-level *TGF-β3* expression in specific cells (Fig. 2C) (15). *TGF-β3* promoter also contains

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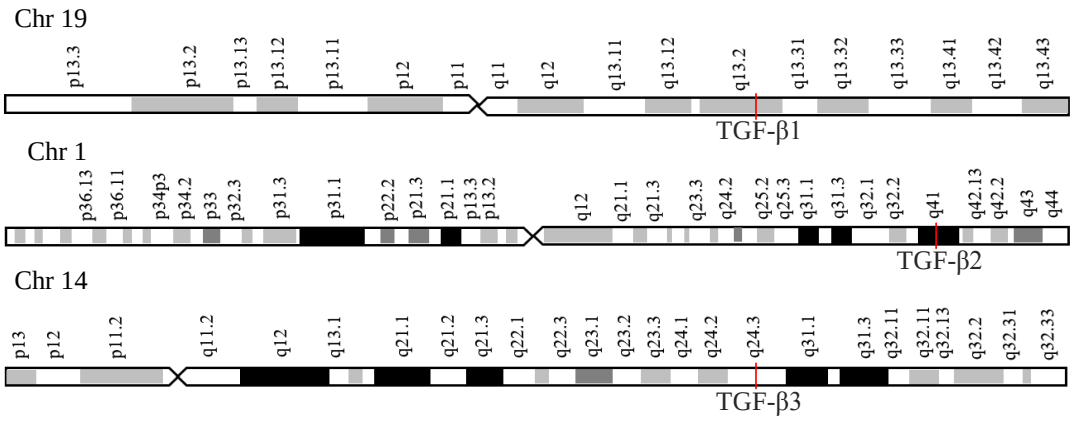

Fig. 1. The chromosomal position of each TGF-β isoform.

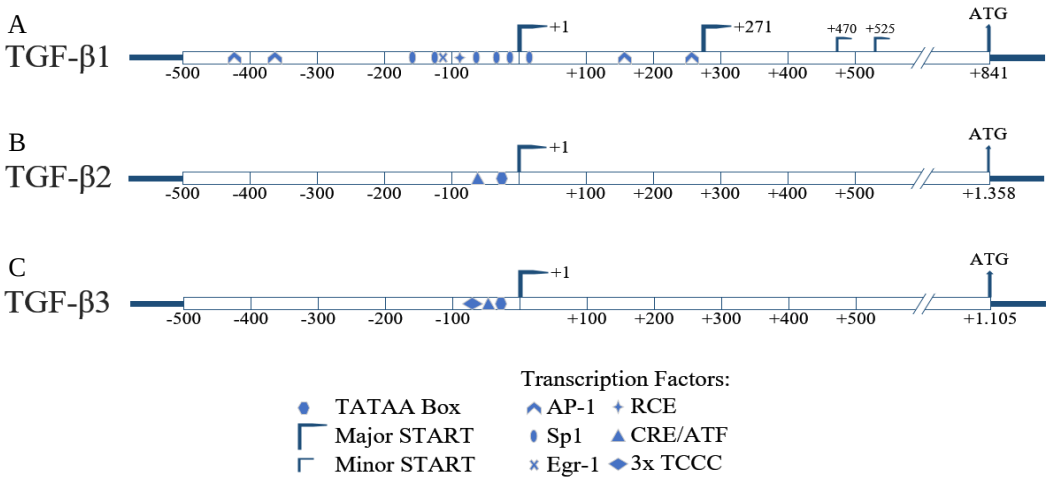

Fig. 2. Gene and protein ribbon structure of TGF-β isoforms. A: *TGF-β1* promoter structure shows no typical TATAA box but contains multiple start points and several transcription factors binding sites; B: *TGF-β2* promoter structure shows typical TATAA box adjacent to the single start point and CRE/ATF binding site; C: *TGF-β3* promoter structure shows typical TATAA box adjacent to the single start point and CRE/ATF binding site with a repetitive TCCC motif addition.

a raloxifene response element (RRE) positioned within -38 to +110 of its sequence. This element mediates the induction of *TGF-β3* in bone by a selective estrogen response modifier (raloxifene) and an estrogen metabolite (17-epiestriol) (16).

TGF-β isoforms have a comparable three-dimensional structure, which consists of a couple of cystine-knotted monomers tied together by a disulfide bond (6). The monomer's shape is described as a spreading hand, and each monomer is arranged in a position in which the palm of one hand faces the heel of the other, forming a homodimer (17, 18). All TGF-β isoforms bind to their receptors with distinct kinetics. Unlike TGF-β1, TGF-β2 and TGF-β3 have been demonstrated to significantly bind to the type I receptor, TβRI. However, in the presence of type II receptor, TβRII, the binding of all three isoforms to TβRI is considerably increased. The differences in affinities and kinetics of their receptor binding may be the basis of the unique biological activities of TGF-β isoforms (19, 20).

TGF-β signaling and regulation

TGF-β can act as a ligand that binds to its receptors for either autocrine or paracrine signaling in response to environmental stimuli (3). In paracrine signaling, TGF-β is produced and secreted by cells as a ligand for receptors of their adjacent cells to mediate cell-cell communications.

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On the other hand, in autocrine signaling, cells signal themselves by releasing TGF-β that binds to its own receptors. TGF-β signaling is regulated by either Smad, non-Smad, or crosstalk between both mediators to induce various physiological and pathological cellular effects (2, 3, 21).

Activation of TGF-β ligands

Each TGF-β isoform is expressed differently in tissues. The amino acid sequence of the three isoforms has up to 70-80% homology. Each TGF-β molecule is synthesized as part of a large precursor molecule which has a pro-peptide part (22). The secreted TGF-β is still in its inactivated form that is known as the latent TGF-β complex containing TGF-β and its latent associated protein (LAP). Together, they are referred to as the small latent complex. The small latent complex is stored in an extracellular matrix to form a complex with latent TGF-β binding protein (LTBP) after being secreted. Linked by disulfide bonds between LAP and LTBP, the three-molecular aggregates complex between TGF-β, LAP, and LTBP is also known as the large latent complex (4, 23). Secretion of TGF-β in its inactive form is paramount for the regulation of its activity. The TGF-β molecule binding to TGF-β latency complexes limits its biological activity by preventing it from binding to its receptors. The release of LAP from LTBP and TGF-β from LAP is an important step that will then initiate the TGF-β activation process. The activated TGF-β is a homodimer molecule that has a molecular weight of 25 kDa (3, 4, 23, 24).

The mechanism underlying the conversion of TGF-β into its biologically active form is not yet fully understood. Various extracellular factors such as extreme acidic or basic pH treatment, heating, and the use of chaotropic agents can activate the latent TGF-β *in vitro* (11). In addition, several cellular factors such as reactive oxygen species, enzymes, α_v-containing integrin, and thrombospondin -1 may also mediate the alteration of LTBP conformity and release the active TGF-β (11). Enzyme activity of plasmin and protease such as matrix metalloproteinase (MMP) can also break the bonds in the latent complex to activate TGF-β. On the other hand, transglutaminase mediates TGF-β bioavailability by anchoring the LTBP to extracellular matrix, thus affects the TGF-β activity (23, 24).

The conformation-difference effect among three TGF-β isoforms on signaling activity has been demonstrated using a chemoregulated migration assay in three-dimensional collagen matrices (17). In cell culture, TGF-β1 and TGF-β3 usually have indistinguishable activity. However, their conformation seems to affect the migration of dermal fibroblasts differently through a matrix of native collagen. TGF-β1 with closed-form structural arrangement has less detectable chemo-regulated migration activity, whereas TGF-β3 with predominantly open conformation shows a potent chemo-regulated migration activity (17).

TGF-β receptors

Most human cells have receptors for TGF-β on their cell surface. There are three types of TGF-β receptors (14). Type I receptor (TβRI) and type II receptor (TβRII) are the two types of receptors that are mainly involved in TGF-β signal transduction. Both have small extracellular domains, single transmembrane domain, and intracellular domains that act as serine/threonine kinase (25). TGF-β will initially bind to TβRII, which then attracts TβRI to form a heteromeric complex. The TβRI will then activate the intracellular signaling pathway by phosphorylating the cytoplasmic signaling component (25). Meanwhile, type III receptors (TβRIII) are transmembrane proteoglycans that modulate ligand access to the signaling receptors, TβRI and TβRII. TβRIII allows high-affinity bonds between TGF-β2 and TβRII, as TβRII tends to bind TGF-β2 weakly due to low affinity between them. Several studies have shown that in cell lines lacking TβRIII, TGF-β2 is around 100-1000 times less potent than the other two

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isoforms (17, 20). The mechanism of action for TβRIII is not yet fully identified. Although TβRIII seems to be able to increase the TGF-β2 affinity, TβRIII activity does not appear to affect the TGF-β1. However, TβRIII modification in cells expressing oncogenic Kirsten rat sarcoma virus (*K-Ras*) gene appears to influence TGF-β1 signaling. In the absence of TβRIII modification, these cells become resistant to TGF-β1 inhibition (26).

Smad-dependent TGF-β signaling

Smad proteins are the predominant mediators of TGF-β signaling. They have the role of changing, regulating, and linking several signaling inputs primarily from TβRI phosphorylation straight to the target gene regulation and expression. Based on structural features and specific functions, Smads are grouped into three different classes: receptor-regulated (R-Smads), inhibitory (I-Smads), and common-partner (Co-Smads) (25).

The three Smads classes have complementary roles in TGF-β signaling. Following the binding of TGF-β to TβRII, the TβRI is recruited and activated which then phosphorylates the R-Smads (Smads1/2/3/5/8). In general, Smads2/3 facilitates the TGF-β, activin, and nodal signaling, whereas Smads1/5/8 facilitates the BMP signaling. Phosphorylation of R-Smads by the receptors will trigger Co-Smads (Smad4) to integrate and relocate the Smads complex to the nucleus. In the nucleus, the Smad complexes later modulate the target gene expression by playing the transcriptional regulator role at specific DNA sites. I-Smads (Smad6/7) have an antagonistic role in signaling by blocking the association of R-Smads with Co-Smads and directing the ligand-receptor complex to be degraded. This inhibitory activity of I-Smads prevents the continuation of the signaling cascade that regulates the target gene expression (25).

Subcellular location is crucial for Smad activity. Other signaling components can obstruct Smads translocation before they shift into the nucleus. The oncogenic Ras is known to inhibit TGF-β signaling by blocking the Smad2/3 translocation to the nucleus via the MAP kinase pathway. In the nucleus, Smad2/3 variations in the Smad complex also determine the gene expression regulation. Both can influence the transcription rate synergically to one gene but antagonistically to another. The difference in the activity model of Smad2/3 shows that they can have different target genes. Moreover, by changing the composition of other transcription proteins involved in forming the complex, specific transcription regulations can be achieved (25).

The DNA binding factors greatly influence the Smad complex binding to DNA and its gene target modulations. The Smad complex can recognize CAGA (Smad binding element) sequences or some GC-rich sequences in DNA. However, their affinity is relatively weak, thereby requiring various cofactors to strengthen it (27). Depending on the cells or the DNA sequence, the cofactors which assist the Smad complex binding to DNA can vary. They determine the Smad complex's induction or repression effect to the target gene expression. This mechanism explains the different roles of TGF-β signaling in distinct cell types (28).

Non-Smad-dependent and crosstalk TGF-β signaling

TGF-β can also activate various signaling pathways independent of Smad proteins. It can induce PI3K/AKT, Rho GTPase, MAPK, and p38/JNK pathways to promote various biological effects on cells. The dual function of TGF-β as a cell growth inhibitor and epithelial-mesenchymal transition (EMT) promoter can also be done through mammalian target of rapamycin (mTOR) and PI3K/AKT signaling. Furthermore, TGF-β can also use Rho GTPase and MAPK signaling pathways to induce the EMT process (28).

Smad proteins can also function as a platform for the crosstalk signaling mechanism. Protein kinase C can interact directly and phosphorylate

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Smad3 to prevent it from binding to DNA (29). Through induction of Smad7 expression, signal transducer and activator of transcription (STAT) and nuclear factor kappa B (NF-κB) signaling can increase the I-Smads activity and inhibit TGF-β signaling (30). The TGF-β signaling pathway is also known to collaborate with p53 and Wnt signaling pathways in modulating TGF-β target gene expression (31).

The Ras signaling pathway shows unique contradictory interactions with TGF-β signaling. Through the Ras route, extracellular signal-regulated kinase (ERK) can phosphorylate the connecting regions in Smad1/2/3 and prevent them from translocating into the nucleus (32). Therefore, cells with high Ras activity are no longer sensitive to TGF-β stimulation. However, there are reports showing that ERK increases the half-life in cells with Ras transformation. It increases the transcriptional activity mediated by the Smads complex by enforcing the complex formation stability between R-Smads and Co-Smads (25). The various models and stages involved in the crosstalk between the Smad and non-Smad pathways show a highly systematic complexity in the TGF-β signaling regulation. The interaction context of the microenvironment with the cells and a precise cell's specific regulatory process is critical in understanding the physiological and pathological relevance of the TGF-β signaling (33, 34).

The role and target gene of TGF-β signaling

TGF-β is implicated in a wide range of biological processes including embryonic development, immune system modulation, cell proliferation, cell differentiation, wound healing processes, and angiogenesis (10-13). The complexity of TGF-β activity is often illustrated by its ability to initiate contradictory effects. For example, while TGF-β is a potent growth inhibitor, it can stimulate cell growth in specific tissues (33).

TGF-β also controls the production of various components of the extracellular matrix. TGF-β promotes the synthesis of thrombospondin, tenascin, collagen, fibronectin, and proteoglycans. Moreover, it also stimulates plasminogen activator inhibitor-1 and tissue inhibitors metalloprotease-1 synthesis. These abilities make TGF-β capable of mediating deposition of the extracellular matrix and allow its remodelling which affects various processes from morphogenesis to cell apoptosis (35).

As stated earlier, despite its name as a growth factor, TGF-β can also be a potent inhibitor of cell growth. The ability of TGF-β to trigger cytostatic and apoptotic effects enables its role as a tissue growth suppressor. This function can be found mainly in the stromal, hematopoietic, and typical epithelial and endothelial tissues (33, 34). Unfortunately, under specific cellular context, like in late carcinogenesis, the inhibitory activity of TGF-β may switch to become stimulatory activity instead. This dual role of TGF-β in cancer gave rise to the term of TGF-β paradox. More information on the TGF-β paradoxical role in cancer and its related genes can be seen in Table 1 (36, 37).

Regulation of TGF-β production

There are complex regulation processes involved in each of the TGF-β production steps. In many cell types, TGF-β synthesis seems to be regulated independently at the level of gene transcription, mRNA processing, mRNA translation, and its secretion. Moreover, the mechanisms leading to particular regulation of TGF-β synthesis might dependt on the type of stimuli and cells (38).

Some cells amplify the TGF-β production via the Ras and MAPK pathways. Ras activation induces downstream signaling cascades through both MKK4/SAPK and MEK1/ERK pathways. These pathways later regulate specific AP- 1 proteins, JunD and Fos/Fra-2, thereby stimulating the complex protein formation at the AP-1 site in the *TGF-β* promoter (39, 40). Interestingly, TGF-β predominant mediator- proteins, Smad3/4, also

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Table 1. Effect of TGF-β signaling on target genes.

Role	Impact	Target gene	Regulation
Tumor Suppressor	Inhibits cell proliferation	*p15, p21, p57, 4EBP1* *CDC25A, E2F-1, Id1-3, c-MYC*	Up Regulated Down Regulated
	Induces apoptosis	*BIK, BIM, DAPK, Fas, GADD45β* *BCL-XL, BCL-2*	Up Regulated Down Regulated
	Activates autophagy	*ATG5, ATG7, Beclin 1/ATG6, DAPK*	Up Regulated
	Inhibits growth factors in the tumor stroma	*HGF, MSP, TGF-α*	Down Regulated
	Inhibits angiogenesis	Thrombospondin	Up Regulated
	Supresses inflammation	*FOXP3* *GATA-3, T-bet*	Up Regulated Down Regulated
Tumor Promoter	Promotes cell proliferation	*PDGF-B*	Up Regulated
	Supresses the immune response	*FOXP3* Fas ligand, *GATA-3*, Granzyme A/B, *IPN-γ, MICA, NKG2D, Nkp30,* Perforin, *T-bet*	Up Regulated Down Regulated
	Promotes angiogenesis	*VEGF, MMP-2, MMP-9* *TIMP*	Up Regulated Down Regulated
	Promotes cancer stem cell self-renewal	*LIF, SOX4*	Up Regulated
	Promotes the epithelial to mesenchymal transition	*SNAIL1/2, ZEB1/2, HMGA2*	Up Regulated
	Promotes metastasis	*HDM2, MMP-9*	Up Regulated

appear to contribute indirectly to this mechanism because they do not bind to the relevant AP-1/SBE site in the *TGF-β* promoter. Smads proteins influence partial stimulation of AP-1 complex formation by regulating c-Jun expression as one of the AP-1 heterodimer complex (41). Therefore, the AP-1 protein complex will induce *TGF-β* transcription (41).

In diabetic neuropathy pathogenesis, the TGF-β1 isoform has been claimed to be responsible for the development of renal tubulointerstitial fibrosis (42). A high concentration of glucose is known to stimulate TGF-β1 production within the glomerulus. However, through p38 MAP kinase activation, glucose can only induce *TGF-β1* transcription and increase cell sensitivity towards platelet-derived growth factor (PDGF) in renal proximal tubular epithelial cells. A significant increase of newly synthesized TGF-β1 can only be achieved after the subsequent addition of PDGF (43, 44).

In apoptotic cells, *TGF-β* mRNA expression and protein synthesis are also separately regulated (38). Pathways like ERK and JNK have been shown to be involved in *TGF-β* transcription regulation. Blockade to ERK and JNK phosphorylation can inhibit phospho-c-jun, which is one of the AP-1 complex that binds to a promoter to upregulate *TGF-β* expression (38, 41). However, TGF-β translation seems to be regulated through RhoA pathway, and its downstream signaling cascade is independent of ERK and JNK signaling pathways. RhoA pathway activates AkT and eukaryotic translation initiation factor (eIF4E) to initiate TGF-β translation (38). eIF4E is the cap-binding protein that binds to mRNA, allowing the recruitment of ribosomes and initiation of translation (45). The activity of PI3K/AkT following RhoA activation is thought to involve mTOR signaling to phosphorylate eIF4E-binding protein 1 (4E-BP1) and cause its release from eIF4E to enable cap-dependent translation to start (38).

TGF-β autoinduction

The TGF-β signaling pathway is also known to be able to self-induce its ligand production. Induction of *TGF-β* expression by the TGF-β

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signaling pathway is referred to as autoinduction. Through this positive feedback loop, specific cell types express *TGF-β* after being exposed to exogenous TGF-β stimulus. This autoregulatory ability may be responsible for prolonging and amplifying the effects of TGF-β on the cells and their microenvironments. It is especially critical during the developmental stage or in pathological processes like carcinogenesis (46).

Not all cells that are responsive to TGF-β will respond to exogenous TGF-β stimulus by autoinduction. Research using endothelial cell line of the fetal bovine heart has shown that TGF-β exposure cannot trigger autoinduction even though the cells are sensitive to TGF-β signaling (47). A distinct TGF-β autoinduction model observed in rat heart fibroblasts and myocytes has been shown to affect the increase of TGF-β synthesized by both cell types. Nonetheless, the production level, the length of production time, and the lag time for the production to start after exposure between them are different (48). Administration of TGF-β triggered an increase in the *TGF-β* expression of rat heart fibroblasts of more than 3-fold in fewer than 4 hours after the exposure. However, this increased expression only lasted for a moment then fell under the control levels within 8 hours after exposure. Meanwhile, in rat heart myocytes, autoinduction occurred slower but for a longer duration. Exposing rat heart myocytes to exogenous TGF-β optimally triggered an increase of *TGF-β* expression within a range of 7 to 24 hours. The rise in *TGF-β* expression of rat heart myocytes was around 2-fold. However, the increase in expression was relatively more stable, as the expression level decreased relatively slowly. It only fell below the control levels 48 hours after the exposure (48).

Exogenous TGF-β exposure experiments on human pulmonary fibroblast cells also reported the autoinduction of TGF-β signaling (49). Induction for 4 hours of human lung fibroblast cells using exogenous TGF-β successfully triggered an increase in *TGF-β* expression of 3-4 fold. It was also followed by the rise of endogenous TGF-β secretion. Exposure to TGF-β in a relatively short time against human pulmonary fibroblast cells was able to provide autoinduction effects with quite a long duration. Increased expression of *TGF-β* by human lung fibroblasts due to autoinduction occured within the range of 90 minutes to 72 hours after induction. Exogenous TGF-β induction was also known to stimulate TGF-β secretion in human pulmonary fibroblasts. However, it did not happen simultaneously with the mRNA overexpression. There was a pause of several hours between the rise of mRNA and the increased amounts of TGF-β secretion (49).

The time gap between the rise of mRNA and the protein secretion of the TGF-β1 isoform has also been demonstrated in renal proximal tubular epithelial cells (43). The study showed that *TGF-β1* expression in human renal proximal tubular epithelial cells immortalized by transduction with human papilloma virus 16 E6/E7 genes (HK-2 cells) increased after recombinant TGF-β1 treatment. It occurred from 3 to 48 hours of the incubation period. Increased *TGF-β1* expression in these cells could reach up to 2.5-fold compared with controls. The rise of *TGF-β1* mRNA was then followed by an increase in protein synthesis within 48 to 72 hours (43).

The increase in TGF-β1 concentration on renal tubular epithelial cells was significantly higher in wild-type cells than in cells without Smad3 (50). This study suggested the involvement of Smad3-mediated signaling in the autoinduction mechanism. Mice without Smad3 also exhibited failure of the TGF-β1 amplification process in injuries that could usually increase TGF-β1 through autoinduction positive feedback loops. It is similar to the report on fibroblasts and monocytes. Significantly reduced monocyte levels in kidneys without Smad3 undergoing unilateral ureteral obstruction (UUO) also appear to affect endogenous TGF-β1 synthesis.

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The reduction of monocytes in this injury suppresses TGF-β1 concentrations around renal tubular epithelial cells, and this indirectly reduces the autoinduction stimulus and contributes to the decrease in TGF-β1 levels after UUO (50).

Regulation of TGF-β autoinduction

TGF-β utilises several pathways to support its autoinduction (Fig. 3). Two *TGF-β* promoter regions are responsive to autoregulation. The first is at the end of the 5' upstream transcription initiation site. The second is between the two main spots for transcription initiation. In both regions, the AP-1 complex binding mediates autoinduction. Moreover, TGF-β signaling also contributes to the expression of Jun family including c-Jun. The heterodimer complex of c-Jun and c-Fos forms the AP-1 transcription factor that binds to the *TGF-β* promoter (41, 51).

Control of the AP-1 protein stability will critically affect the mechanism of autoinduction. The Lin11, Isl-1, and Mec-3 (LIM) protein domain only 7 (LMO7) can inhibit AP-1 activity (51). LMO7 can facilitate ubiquitination and proteasomal degradation of the AP-1 subunits. As a result, LMO7 can block the autoinduction process and eventually downregulates TGF-β. LMO7 itself is also induced by TGF-β via canonical signaling pathway, but only after the TGF-β concentrations are high enough and the signaling has occurred for a sufficient duration. Therefore, LMO7 acts as a sensor and a negative feedback regulator of TGF-β signaling pathway. LMO7 activity is also known to affect Smad3, thus involved in the regulation of TGF-β target gene expression. However, the influence of LMO7 on the TGF-β non-canonical signaling pathway still needs to be further explored (51).

Furthermore, the non-Smad signaling pathway also regulates TGF-β1 autoinduction in the proximal tubules of the kidney. Pathways like ERK

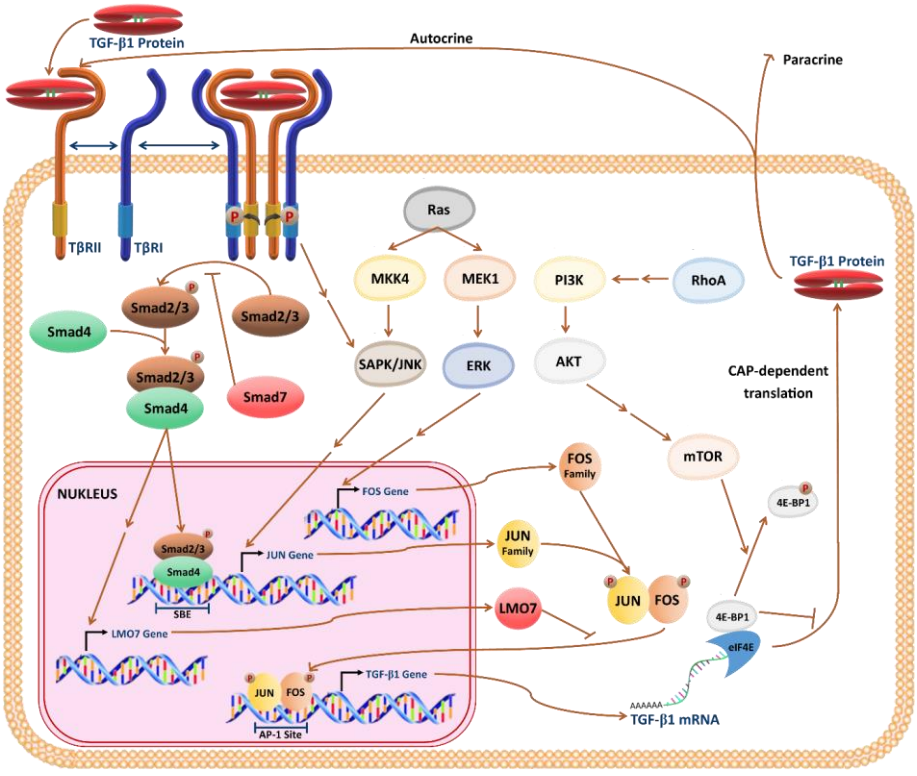

Fig. 3. Known regulators of TGF-β autoinduction. In this illustration, TGF-β1 isoform is used as an example of the ligand being self-induced. Smad and JNK pathways induce expression of JUN family protein as an AP-1 component. Together with the FOS family protein induced by the ERK pathway, JUN family protein form an AP-1 complex that promotes *TGF-β1* transcription. Later, RhoA-mTOR pathways enable TGF-β1 protein translation through phosphorylation and dissociation of 4E-BP1 from eIF4E. LMO7 inhibits AP-1 transcriptional activity and acts as a negative feedback regulator to prevent further TGF-β1 production.

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MAP and p38 MAP are known to influence the regulation of the TGF-β1 autoinduction mechanism. Evidence has shown that ERK MAP suppresses the induction of TGF-β1 expression, while inhibition of p38 MAP negatively affects TGF-β1 protein synthesis (43).

The role of TGF-β autoinduction in developmental and pathological processes

The pulmonary fibroblasts are known to display TGF-β autoinduction (49). This mechanism can be a vital factor in determining local pulmonary phenotypes. Pulmonary fibroblasts respond to TGF-β as a mitogen or as an inducer of gene expression associated with the extracellular matrix (52). Additionally, mesenchymal, smooth muscle, and bronchiolar epithelial cells can also act as primary sources of TGF-β for the pulmonary fibroblasts (49). Upregulation of *TGF-β* by resident interstitial fibroblasts may increase local TGF-β concentrations during the pulmonary developmental and repair process (53).

The cardiac fibroblasts and cardiomyocytes are also known to exhibit TGF-β autoinduction. It maintains the increase of TGF-β levels observed after a heart attack. Exogenous TGF-β administration also showed similar results by inducing endogenous TGF-β expression to maintain its cardioprotective effect (54). In both conditions, an increase in TGF-β levels triggers tissue repair through the induction of angiogenesis, extracellular matrix production, and inflammatory cell recruitment and activation (55). In neonatal cardiac fibroblasts, the autoinduction of *TGF-β* occurs quite rapidly. The degranulated platelets may release a certain amount of TGF-β ligand at the site of injury that acts as a trigger for temporary autoinduction activity (48).

Not only is TGF-β implicated in developmental and physiological processes, it is also involved in various pathological processes. For example, as mentioned earlier, TGF-β1 autoinduction also affects patients with UUO. TGF-β1 autoinduction has been thought to worsen the severity of this kidney disease. Elevated levels of TGF-β1 in UUO patients initially occur as a response to damage to the tubules due to pressure from urine (50). However, if left uncontrolled, the accumulation of TGF-β1 in the kidney tubules can cause chronic inflammation in the interstitial tubular tissue (56). TGF-β1 can also encourage EMT and extracellular matrix accumulation. The EMT will transform the renal tubular epithelium into myofibroblasts. The autoinduction of TGF-β1 then continues as part of a positive feedback loop in the injured area. It continuously increases TGF-β1 levels that support the process of fibrogenesis in the renal tubules. Ultimately, this persistent fibrogenesis will lead to irreversible damage that promotes organ failure (50).

The role of TGF-β autoinduction in cancer

The TGF-β expression has already been studied in almost all epithelial cancers such as lung and breast cancers (57). In benign epithelial and other early-stage tumors, TGF-β is an excellent growth suppressor. The ability of TGF-β to inhibit cell growth underlies the TGF-β tumor suppressor trait. Nevertheless, tumor cells often acquire the ability to avoid the TGF-β anti-proliferation effects. These changes can be in the form of mutations in essential components of the TGF-β signal transduction cascade. These mutations cause the TGF-β signaling pathway to malfunction (58). Moreover, in advanced tumors, the TGF-β signaling pathway itself can turn ominous and no longer inhibits carcinogenesis. Instead, it stimulates tumor growth and development. Under this circumstance, TGF-β autoinduction may result in devastating outcomes (59).

An experiment in Ha-Ras transformed epithelial cells showed that the TGF-β autoinduction supports the progression of tumor tissue to become more invasive. Ha-Ras oncoprotein is known to work with endogenous TGF-β to induce the transformation of mammary

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epithelial cells. Together, they support the development of invasive properties both *in vitro* and *in vivo* tumor formation in mice. Non-tumorigenic mammary epithelial cell cultures or those undergoing Ha-Ras transformation without TGF-β treatments show single layer cell growth with a typical structure. However, TGF-β addition to the culture medium triggers the Ha-Ras transformed cells to develop more invasive traits. They appear to be resistant to the TGF-β growth inhibition effects. In contrast, the non-tumorigenic mammary epithelial cells appear to experience a growth setback and increased apoptosis after the same treatment (60).

In addition to changing its nature to be more invasive, the Ha-Ras transformed epithelial cells also start producing TGF-β independently. Tests on the TGF-β activity disruption by administering TGF-β antibodies to the cell culture medium resulted in invasive trait suppression and cell nature recovery (60). It shows the ability of TGF-β to both induce the invasive properties and maintain them via an autocrine feedback system.

The plasticity of cell properties under TGF-β influence is an invasive cell's common trait that can explain the cancer cell's ability to acquire fibroblast cell-like migrating capability. These traits also support the possibility of different organ's microenvironment to induce the invasive cells to come out of blood vessels into specific organs and regain non-invasive characteristics (60).

TGF-β as a potential therapeutic target

Taking into account the involvement of TGF-β signaling pathway and autoinduction in various pathological conditions such as cancer, TGF-β has increasingly been studied as a potential therapeutic target. Several drugs that target components of the TGF-β signaling pathway have been developed. The mechanisms of action of these drugs include preventing transcription of the *TGF-β*, marking *TGF-β* mRNAs for degradation, and hindering ligand-receptor interaction. Clinical trials have been conducted for certain drugs targeting the TGF-β pathway for cancer, such as antisense RNAs, therapeutic antibodies against TGF-β ligands or receptors, vaccines, and small molecule kinase inhibitors. However, limited understanding about the dual role of TGF-β as a tumor promoter and tumor suppressor poses a problem for the development of these drugs (61, 62). A number of TGF-β targeted therapies have also been developed for the treatment of fibrosis, which mainly work by either dampening the pro-fibrotic activity of TGF-β signaling pathway or increasing the anti-fibrotic activity of BMPs (63). In addition, directly modulating the mediator and regulator proteins of the TGF-β autoinduction process can also become an alternative approach. The use of a negative regulator such as LMO7 or inhibition to the known upstream pathway mediators like Smad3, ERK, and JNK might be utilised as a model to restrict TGF-β autoinduction (43, 51). Targeting the AP-1 as a direct regulator of TGF-β autoinduction can also disrupt the malfunctioning TGF-β in pathological conditions (41, 51). Nevertheless, future studies are needed to provide more insight into the TGF-β autoinduction mechanism before further therapeutic approaches could be developed.

Conclusion and future perspectives

The TGF-β signaling pathway is involved in many biological reactions which include both physiological and pathological processes. The diverse effects of TGF-β isoforms in specific cells and circumstances are driven by the differences in spatial-temporal expression patterns of TGF-β family members. The complexity of TGF-β signaling lies in the regulation of related mediators and each step of the signaling cascade as well as the crosstalk between multiple related pathways. Therefore, cells control the TGF-β signaling meticulously to meet the specific cellular and microenvironmental interaction context. Interestingly, the TGF-β pathway has been found to be able to self-induce its ligand production in a

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process known as autoinduction. It utilises several pathways to support this autoinduction process, which include the Smad, JNK, ERK, and RhoA-mTOR pathways (Fig. 3). TGF-β might be known as an essential growth factor in the morphogenesis and developmental state, but in carcinogenesis TGF-β is also known to help tumor cells advance and acquire more aggressive traits. It is also implied that TGF-β autoinduction also affects and modulates migrating cancer cell properties. Furthermore, TGF-β autoinduction is also thought to increase the severity of other pathological conditions that may even lead to organ failure. These insights have led to the idea that TGF-β and its autoinduction could be targeted as a potential therapeutic target for various pathological conditions, particularly cancer. In conclusion, the field of TGF-β autoinduction is still understudied, and this paper provides early insight into its regulation, signaling, and clinical importance. Future research is needed to better understand the mechanism of autoinduction for TGF-β in pathological conditions as well as how to target it for therapeutic purpose.

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Conflict of Interest

The authors declare no competing interests.

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