

Exploring TGF- β Signaling in Cancer Progression: Prospects and Therapeutic Strategies

Khansa Ali Sheikh^{1,*}, Momna Amjad^{1,*}, Mahnoor Tabassum Irfan^{1,*}, Sumaira Anjum¹,
Tanveer Majeed¹, Muhammad Usman Riaz², Amar Yasser Jassim³, Elham Abdullatif M Sharif⁴,
Wisam Nabeel Ibrahim⁴

¹Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan; ²School of Computer Science, University College Dublin, Belfield, Dublin 4, Ireland; ³Marine Science Center, University of Basrah, Basrah, Iraq; ⁴Department of Biomedical Sciences, College of Health Sciences, QU Health, Qatar University, Doha, Qatar

*These authors contributed equally to this work

Correspondence: Sumaira Anjum, Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan, Tel +923006957038, Email sumaira.anjum@kinnaird.edu.pk; Wisam Nabeel Ibrahim, Department of Biomedical Sciences, College of Health Sciences, QU Health, Qatar University, Doha, Qatar, Email w.ibrahim@qu.edu.qa

Abstract: Cancer persists as a ubiquitous global challenge despite the remarkable advances. It is caused by uncontrolled cell growth and metastasis. The Transforming Growth Factor-beta (TGF- β) signaling pathway is considered a primary regulator of various normal physiological processes in the human body. Recently, factors determining the nature of TGF- β response have received attention, specifically its signaling pathway which can be an attractive therapeutic target for various cancer treatments. The TGF- β receptor is activated by its ligands and undergoes transduction of signals via canonical (SMAD dependent) or non-canonical (SMAD independent) signaling pathways regulating several cellular functions. Furthermore, the cross talk of the TGF- β signaling pathway cross with other signaling pathways has shown the controlled regulation of cellular functions. This review highlights the cross talk between various major signaling pathways and TGF- β . These signaling pathways include Wnt, NF- κ B, PI3K/Akt, and Hedgehog (Hh). TGF- β signaling pathway has a dual role at different stages. It can suppress tumor formation at early stages and promote progression at advanced stages. This complex behaviour of TGF- β has made it a promising target for therapeutic interventions. Moreover, many strategies have been designed to control TGF- β signaling pathways at different levels, inhibiting tumor-promoting while enhancing tumor-suppressive effects, each with unique molecular mechanisms and clinical implications. This review also discusses various therapeutic inhibitors including ligand traps, small molecule inhibitors (SMIs), monoclonal antibodies (mAbs), and antisense oligonucleotides which target specific components of TGF- β signaling pathway to inhibit TGF- β signaling and are studied in both preclinical and clinical trials for different types of cancer. The review also highlights the prospect of TGF- β signaling in normal physiology and in the case of dysregulation, TGF- β inhibitors, and different therapeutic effects in cancer therapy along with the perspective of combinational therapies to treat cancer.

Keywords: transforming growth factor beta, canonical signaling pathway, tumor promoting, tumor suppression, Hedgehog, cancer, combinational therapies

Introduction

Overview of Cancer

One of the global challenges we face today is cancer. It is because cancer is causing suffering on a global scale by affecting people of all ages. Despite the advances in science and technology, the projections of cancer cases are still increasing. According to an estimate made by the International Agency for Research on Cancer (IARC), there are expected to be around 26 million new cancer cases and 17 million deaths due to cancer globally by 2030.¹ In cancer, cell growth is uncontrolled and it spreads through different routes throughout the entire body with a process referred to as metastasis.² The basic hallmarks of cancer are progression through activation of invasion and metastasis processes,

sustained proliferative signaling, stimulation of angiogenesis, evasion of growth suppressors, permitting replicative immortality, and resistance to cell death.³ Genetic and environmental factors cause cancer. Genetic factors involve a spectrum of somatic and germline mutations affecting different aspects of cell survival and viability processes. In contrast, environmental factors trigger these mutations by various chemical or physical agents.⁴ In addition to that, aging is a vital and critical risk factor in identifying and managing cancer during its initial stages.⁵ Due to aging, there is a decline in tissue health and intracellular communication by which various signals are ignored. In this way, tumors are formed because the cells ignore the signal transduction process. Among the myriad of molecular mechanisms inducing cancer progression, the Transforming Growth Factor-beta (TGF- β) signaling pathway stands out as a significant player, exhibiting dual roles in both tumor suppression and promotion of cancer, depending on the type and stage of the disease.⁶ The TGF- β conflicting effects based on the tumor stage continue to cast doubt on its involvement in cancer. In this review article, we intend to explore the signaling pathways involved in cancer and specifically examine TGF- β role in cancer treatment, highlighting various clinical trials and combination therapies.

TGF- β in Normal Physiology

Biological signals in multicellular organisms serve as crucial coordination and communication pathways, essential for these organisms' proper functioning, growth, and development.⁷ These signals are essential for the proper regulation and homeostasis of living organisms. TGF- β family comprises pleiotropic cytokines and signaling molecules with diverse roles across the animal kingdom. While cytokines typically mediate cell communication and immune responses, TGF- β carries out a diverse range of biological processes in both embryonic and adult stages of life. This includes differentiation, wound healing, proliferation, and regulation of cell and tissue-specific motility.⁸

TGF- β consists of a family of ligands including TGF- β I, TGF- β II, and TGF- β III.⁹ These ligands are TGF- β isoforms and are closely related to bone morphogenic proteins (BMPs), activins and various growth and differentiation factors (GDFS). These factors are soluble and have tissue-specific effects. They interact with cell membrane receptor complexes when they are activated, which results in activating cellular responses. The fundamental role of TGF- β lies in maintaining cell and tissue homeostasis through multiple levels of regulated signal transduction. Examples of this regulation include extracellular antagonists, co-receptor molecules, and intracellular regulators.¹⁰ These regulators have garnered significant interest from cancer biologists due to their pivotal roles in critical biological and cellular processes such as embryonic development, cytoskeletal organization, cellular homeostasis and tissue regeneration.¹¹ Disruptions in the TGF- β signaling pathway result in a wide array of pathological issues including cancer, fibrosis and immune diseases.¹² The intricacy of TGF- β signaling is underscored by its activation through multiple mechanisms. Integrins can mediate TGF- β activation by interacting with latent TGF- β complexes, facilitating their conversion to the active form. Acids and bases can alter the local microenvironment, promoting conformational shifts and releasing active TGF- β . Reactive oxygen species (ROS) can cause oxidative modifications, impacting the availability and activity of TGF- β . Thrombospondin-1 (TSP-1) which acts as a significant modulator by binding to latent TGF- β and activating it. Proteases, such as matrix metalloproteinases, can cleave latent TGF- β complexes, releasing the active cytokine.⁸ TGF- β signaling pathway plays an intrinsic role in physiological processes. These processes are as follows:

Cell Growth and Proliferation

The contribution of TGF- β signaling in cell growth and proliferation is multifaceted, while also having significant impacts on cell cycle. TGF- β induces cytostasis, which either upregulates or downregulates cell proliferation in accordance with the cellular context, ultimately causing cell cycle arrest.¹³ This arrest is mediated through two primary mechanisms: (i) regulation of cell cycle inhibitors and (ii) downregulation of c-Myc protein. First, the expression of cell cycle inhibitors, such as p15, p21, and p27, is upregulated by TGF- β signaling.⁸ These inhibitors bind to cyclin-dependent kinases (CDKs) and inactivate them. These enzymes are crucial and drive the progression of the cell cycle from the G1 phase to the S phase. The CDK inhibition ultimately interrupts the cell cycle at the G1 phase, preventing further division of cells. Secondly, c-Myc protein expression is decreased by TGF- β signaling. c-Myc is a potent transcription factor promoting cell cycle progression by driving the gene expression of those required for DNA synthesis and cell division.¹⁴ By downregulating c-Myc, TGF- β ensures that cells do not proliferate unchecked. Additionally, the

TGF- β pathway also plays diverse roles in various cell types. For instance, studies have demonstrated its association with β cell proliferation and development, highlighting the pathway's crucial function in maintaining cellular homeostasis and hindering uncontrolled cell growth, which is characteristic of cancerous tissues. The dual regulatory nature of TGF- β of promoting cytotaxis and controlling cell cycle progression underscores its importance in both normal physiological processes and the pathogenesis of diseases, ie, cancer.¹⁵

Differentiation

TGF- β signaling plays a crucial part in cell differentiation and specialization by shaping the growth and development of numerous cells through intricate molecular mechanisms. One of its significant functions is the conversion of mesenchymal cell differentiation into myofibroblasts. This process involves the activation of SMAD-dependent (canonical) and SMAD-independent (non-canonical) signaling pathways, which regulate gene expression associated with myofibroblast markers, ie, α -smooth muscle actin (α -SMA).^{16,17} Myofibroblasts are essential for tissue healing and fibrosis, contributing to wound contraction and extracellular matrix deposition. Additionally, TGF- β signaling influences the differentiation of precursor cells into chondrocytes and osteoblasts, which are cartilage and bone-forming cells, respectively. In osteoblast differentiation, TGF- β activates the SMAD pathway, leading to the transcription of Runx2 which acts as a critical transcription factor for osteoblastogenesis.¹⁸ Concurrently, TGF- β regulates Sox9 expression, another transcription factor vital for chondrocyte differentiation and cartilage formation. These pathways ensure the proper growth and maintenance of skeletal tissues.

TGF- β also promotes the development and differentiation of immune cells (T and B cells). In the context of T cells, TGF- β signaling is pivotal in inducing regulatory T-cells (Tregs). It does so by upregulating Foxp3 expression which is a transcriptional factor necessary for the formation and proper functioning of Treg.¹⁹ Tregs are involved in maintaining immunological tolerance and preventing autoimmune responses. Similarly, TGF- β influences B cell differentiation into regulatory B cells (Bregs) and certain plasma cell types.²⁰ Bregs, characterized by the expression of IL-10, contribute to immune homeostasis by suppressing inflammatory responses.²¹ Furthermore, TGF- β signaling is involved in mucosal immunity, where it supports the development of IgA-secreting plasma cells, essential for mucosal defense.²² The precise regulation of these differentiation processes by TGF- β ensures a balanced immune response and maintenance of tissue integrity, highlighting its multifaceted role in cellular differentiation and specialization.

Immune Regulation

TGF- β also has an impact on the regulation of the immune system through its potent immunosuppressive effects, mediated by intricate molecular mechanisms. As a central immunosuppressive pathway, TGF- β signaling modulates the activity of various immune cells, ie, T and B-lymphocytes and natural killer (NK) cells. It also regulates T-lymphocytes by promoting the differentiation of Tregs, essential for maintaining immunological tolerance and preventing autoimmune responses. This process involves the activation of SMAD2/3 signaling pathway, which facilitates the transcription of Foxp3 gene referred to as a master regulator of Treg development and function.¹⁹ TGF- β also inhibits the rapid division and effector functions of conventional T-helper cells and cytotoxic T-lymphocytes by interrupting the pro-inflammatory cytokines (IL-2 and IFN- γ) expression, thus curbing excessive immune activation.²¹

In B-lymphocytes, TGF- β influences differentiation into regulatory B-cells (Bregs), producing anti-inflammatory cytokines, ie, IL-10 and TGF- β itself, contributing to the suppression of inflammatory responses and promoting immune tolerance.²¹ TGF- β 's impact on NK cells, crucial elements of innate immune system, involves downregulating their cytotoxic activity and cytokine production. NK cells are vital for identifying and destroying virally infected and malignant cells; however, TGF- β signaling diminishes their effectiveness by altering their receptor expression and reducing their cytotoxic potential.²³ Moreover, TGF- β signaling decreases the pro-inflammatory cytokines (TNF- α and IL-6) production, thereby attenuating overall immune responses and promoting tolerance to self-antigens. Through these multifaceted actions, TGF- β maintains immune homeostasis, preventing autoimmunity and ensuring appropriate immune responses while facilitating tissue repair and regeneration.

Extracellular Matrix Production and Remodelling

TGF- β signaling is a major regulatory pathway responsible for the production and remodeling of extracellular matrix (ECM), playing a fundamental role in tissue and cell homeostasis and repair. This pathway influences the synthesis and organization of ECM's different components, ie, collagen, fibulins, fibronectin and proteoglycans.²⁴ TGF- β imposes its effects through both canonical and non-canonical signaling pathways.

Canonical pathway involves TGF- β binding to TGF- β type II receptor (TGF- β RII), which cause activation and phosphorylation of TGF- β type I receptor (TGF- β RI). When TGF- β RI activates, it phosphorylates receptor-regulated SMADs (R-SMADs), primarily SMAD2 and SMAD3. These phosphorylated SMADSs form a complex by binding with the common mediator SMAD4, which migrates to the nucleus where it regulates the transcription of the target genes. The promoter regions of genes encoding ECM proteins are directly binded with this complex, ie, collagen types I and III, proteoglycans and fibronectin, enhancing their transcription and subsequent protein production.²⁵

TGF- β in the non-canonical pathways activates other signaling cascades such as MAPK (ERK, JNK, p38), Rho-like GTPase and PI3K/AKT pathways. These pathways further contribute to ECM regulation by modulating cellular responses, ie, migration, differentiation and proliferation, which are crucial for ECM remodeling. For instance, ERK pathway activation can lead to the phosphorylation of transcription factors like AP-1, which as a result enhances ECM genes expression.²⁶ Moreover, TGF- β signaling modulates the activity of various transcriptional co-regulators. For instance, TGF- β -induced SMAD complexes can form interaction with co-activators such as CBP/p300, which possess histone acetyltransferase (HAT) activity, facilitating chromatin remodeling and transcriptional activation of ECM-related genes.²⁷ On the other hand, TGF- β also activates co-repressors, ie, SnoN and Ski, which inhibit SMAD-mediated transcription, providing a fine-tuned regulatory mechanism for ECM gene expression.²⁸

TGF- β also promotes the production of matrix metalloproteinases (MMPs) enzymes responsible for ECM degradation and tissue inhibitors of metalloproteinases (TIMPs) which inhibit MMP activity. The balance between MMPs and TIMPs is crucial for controlled ECM remodeling, allowing for tissue repair and maintenance without excessive fibrosis.²⁹ TGF- β -induced expression of MMPs involves SMAD3-mediated transcriptional activation and interaction with transcription factors such as SP1. Conversely, TGF- β upregulates TIMPs through SMAD signaling pathways, ensuring a controlled environment for ECM turnover.

Another critical aspect of TGF- β -mediated ECM regulation is its influence on the integrin signaling. Transmembrane receptors like integrins also facilitate cell-ECM interactions and transmit signals from the ECM to the cell interior. TGF- β can enhance certain integrins expression, promoting cell adhesion and migration to ECM.³⁰ This interaction is considered essential for ECM assembly and remodeling. Additionally, integrin-mediated activation of focal adhesion kinase (FAK) can synergize with TGF- β signaling to further regulate ECM gene expression and cellular responses. TGF- β also regulates the synthesis of connective tissue growth factor (CTGF), a downstream mediator that amplifies the effects of TGF- β on ECM production. CTGF expression is induced by SMAD-dependent pathways and plays a role in enhancing the deposition of ECM components and promoting fibroblast proliferation and differentiation.³¹

The enhanced expression of genes encoding ECM proteins leads to increased deposition and structural organization of the ECM, contributing to tissue strength and integrity. However, dysregulation in TGF- β signaling may result in pathological conditions characterized by excessive ECM deposition, such as fibrosis, or insufficient ECM production, such as in certain degenerative diseases. Therefore, TGF- β signaling is pivotal in maintaining dynamic balance of ECM production and remodeling, essential for normal tissue function and response to injury.

Wound Healing

In the case of wound healing process and various cellular activities, the TGF- β pathway also plays an integral role. TGF- β is released by platelets at initial stages of wound healing, which leads to the development of inflammatory cells at the wound site.³² It promotes cells, ie, fibroblasts and keratinocytes movement and proliferation. These cells are essential for the tissue repair process. It also enhances the production of granulation and angiogenesis of tissues which increases the ECM production and further helps in wound healing activity. This can help in the development of advanced therapeutic methods for wound healing.

Angiogenesis

Angiogenesis, a process involving the formation of new blood vessels from pre-existing ones, is critical in development, wound healing and disease. TGF- β exerts a significant part in regulating angiogenesis through its complex signaling pathways modulating the behavior of endothelial cells. Depending on the context and interacting molecules, TGF- β can either inhibit or promote angiogenesis, making its role highly versatile and context dependent.

In the context of promoting angiogenesis, TGF- β signaling induces pro-angiogenic factor expression, most notably vascular endothelial growth factor (VEGF). VEGF is a powerful stimulator in the endothelial cell proliferation and migration which are essential steps in new blood vessel formation. This can be achieved through SMAD-dependent and SMAD-independent pathway activation where it activates transcription of VEGF and other angiogenic genes in the nucleus.³³ This non-canonical signaling pathway is involved in the fine-tuning of angiogenic responses by modulating the stability and translation of VEGF mRNA. Additionally, TGF- β signaling can stimulate the production of other angiogenic factors, ie, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), which synergistically induce endothelial cell proliferation and new vessel formation.³⁴ TGF- β also contributes to the maturation and stabilization of newly formed blood vessels. It regulates the integrins and other adhesion molecule expression on endothelial cells, facilitating their interaction with ECM and pericytes.³⁵

This interaction is crucial for structural integrity and functional maturation of blood vessels. For instance, TGF- β induces integrin $\alpha\beta 3$ expression, which further enhances endothelial cell adhesion and migration of ECM components, thereby supporting the formation of stable vascular structures.³⁶

Interestingly, TGF- β 's role in angiogenesis is context-dependent and can also be inhibitory. In certain settings, particularly in the case of high levels of TGF- β or in conjunction with other signaling molecules, TGF- β can induce anti-angiogenic responses. This involves the upregulation of angiogenesis inhibitors, ie, thrombospondin-1 (TSP-1) and the suppression of VEGF signaling.³⁷ The TGF- β inhibitory effects are mediated via SMAD-independent pathways, such as activation of the p38 MAPK pathway, which can enhance the expression of anti-angiogenic genes and suppression of endothelial cell growth, proliferation and migration.

Furthermore, TGF- β signaling influences the recruitment and differentiation of mesenchymal stem cells (MSCs) and pericytes, which are essential for vessel stabilization and the prevention of excessive angiogenesis. By promoting MSCs differentiation into pericytes and smooth muscle cells, TGF- β aids in the structural support and functional regulation of newly formed vessels, ensuring proper vascular remodeling and homeostasis.³⁸ In the case of pathological conditions for instance cancer, the dual role of TGF- β in angiogenesis becomes particularly evident. Tumors exploit the pro-angiogenic properties of TGF- β in promoting vascularization and sustaining their growth and metastasis. On the other hand, therapeutic strategies targeting TGF- β signaling aim to disrupt its pro-angiogenic effects and inhibit tumor angiogenesis.

Development and Differentiation in Embryogenesis

Another crucial function of TGF- β signaling pathway is its role in growth, development and differentiation during embryogenesis. Embryogenesis, the process beginning with the fertilization of an egg with sperm cell, involves a series of highly regulated events leading to the formation of new organs and tissues. TGF- β signaling has been instrumental in orchestrating these events, ensuring the proper organization, differentiation, and maintenance of cells in the developing embryo.⁸

During early embryogenesis, TGF- β signaling holds a significant role in the formation of the 3 primary germ layers: (i) ectoderm (ii) endoderm (iii) mesoderm. These layers form all tissues and organs in the body. TGF- β signaling is pivotal in inducing mesoderm formation, a process facilitated by Nodal activation, a member of TGF- β superfamily.³⁹ Nodal signaling, in conjunction with SMAD2/3, regulates mesodermal markers, ie, gooseoid and brachyury expression, driving the differentiation of mesodermal progenitors. In addition to mesoderm induction, TGF- β signaling is essential in neural differentiation from ectoderm. The balance between TGF- β and BMPs is crucial for neural development. BMP signaling promotes epidermal fate, while TGF- β signaling via BMP pathway inhibition, favors neural differentiation by inducing neural-specific transcription factors, ie, Sox2 and Neurogenin.⁴⁰ TGF- β signaling also provides a significant role in endodermal differentiation, influencing the development of internal organs such as liver, pancreas and lungs. By regulating transcription factor expression like Sox17 and Foxa2, TGF- β signaling ensures proper formation and patterning of endodermal tissues.⁴¹ Furthermore, TGF- β signaling regulates epithelial-to-mesenchymal transition (EMT) process

critical for the formation of various tissues and organs. EMT is regulated by TGF- β -induced transcription factors, ie, Snail, Slug and Twist, which repress epithelial markers and activate mesenchymal markers, facilitating tissue remodeling and organ development.⁴²

TGF- β Signaling Pathways

Synthesis of TGF- β

TGF- β is as a large, complex and inactive precursor protein synthesized in a rough endoplasmic reticulum (RER). It consists of a signal peptide which includes; (i) large N-terminal pro-domain referred as latency-associated peptide (LAP) preventing TGF- β activation,⁴³ (ii) short mature peptide C-terminal domain.^{44,45} TGF- β and other members of this superfamily are synthesized in dimer form. The pro-domain then assembles into a homodimer via two disulphide bonds linking LAP portions, while the mature TGF- β moieties interact by single disulphide bond⁴⁶ and form a small latent complex (SLC) by binding non-covalently with LAP. A proteolytic cleavage site is located between pro and mature domains. The bond between pro-domain LAP and short domain is cleaved with the help of convertase furin located in trans-Golgi.^{47,48} The LAP proteins then enfold mature domain which form small latent complex (SLC) by non-covalent bonds and protect the binding of mature TGF- β with its receptors. SLC forms a large, inactive complex as it interacts by with latent TGF- β binding molecule (LTBP) by disulphide bond which is a glycoprotein that acts as TGF- β chaperone and mediates its folding and secretes it into extracellular matrix (ECM).

Activation of TGF- β

For binding with the receptor, the latent TGF- β needs to get activated. Different processes are observed to activate TGF- β . Latent TGF- β activation occurs when mature TGF- β portions are dissociated from the LAP portions. The cleavage may operate in both in vitro and in vivo. The in vitro cleavage includes heating of TGF- β with mild acid lowering the pH to 4.5^{49–51} or by oxidative modification where reactive oxygen species (ROS) cause loss of ability in LAP to bind with mature TGF- β .^{52–54} The in vivo cleavage includes the proteolytic cleavage of LAP via various ECM serine proteases, ie, plasmin, Leucine-rich repeat consisting protein 33 (LRRC33), matrix metalloproteinases (MMPs), ie, MMP9 and MMP14, Cathepsin D and thrombospondin-1 (TSP-1) release the active TGF- β .^{49,55–58} Additionally, LTBP can be associated with LAP by covalent bonding forming a large latent complex (LLC) and deposits SLC in the ECM.^{48,59–61} LLC then covalently binds with a particular ECM protein (fibrillin and fibronectin) through LTBP in a large N-terminal domain.^{59,62,63} Furthermore, LTBP is associated with glycoprotein A repetition predominant protein (GARP), a transmembrane protein of various cells, ie, regulatory T (Treg), endothelial and platelets which activate latent TGF- β .^{64,65} Epithelial restricted integrins, which are cell adhesion receptor proteins, play a part in invasion, proliferation and survival migration of cells while also activating latent TGF- β .^{66–68} Integrins comprises heterodimeric α , β subunits ($\alpha\beta6$, $\alpha\beta8$) which are known as transmembrane receptors type I and found in a variety of different cells.⁶⁶ It has been observed that some integrins also bind to Arg-Gly-Asp (RGD) which is a motif of LAP and generates a mechanical force that deforms the structure of LLP and undergoes cellular contractions that releases active TGF- β .^{47,69–72} The active TGF- β half-life is faster than that of latent TGF- β and if its receptor is absent, then it can be cleared rapidly from the ECM.⁷³ Once latent TGF- β is activated, it controls the timing and location of TGF- β signaling.

Canonical and Non-Canonical TGF- β Signaling Pathways

TGF- β is a versatile, pleiotropic, multifunctional cytokine belonging to a superfamily having ubiquitous cell growth factors such as activins, Bone Morphogenic Proteins (BMPs), inhibitions and anti-Mullerian hormone,⁵¹ expressed in mammals in three isoforms: TGF- β I, II, and III. TGF- β I is considered to be the most abundant and ubiquitously expressed in humans among all, and all three isoforms show 75% of homology via the same receptor complex. TGF- β undergoes transmission of signals through canonical or non-canonical pathways as demonstrated in Figure 1.⁷⁴

TGF- β receptor complex, a tetramer that is comprised of two paired transmembrane serine/threonine protein kinases; 2 T β RI (ALK 1) and 2 T β RII.^{76,77} Betaglycan is the third type of TGF- β receptor (T β RIII) which is a low affinity, non-signaling, co-receptor abundant on different cell surfaces binding TGF- β ligands to high-affinity TGF- β receptor

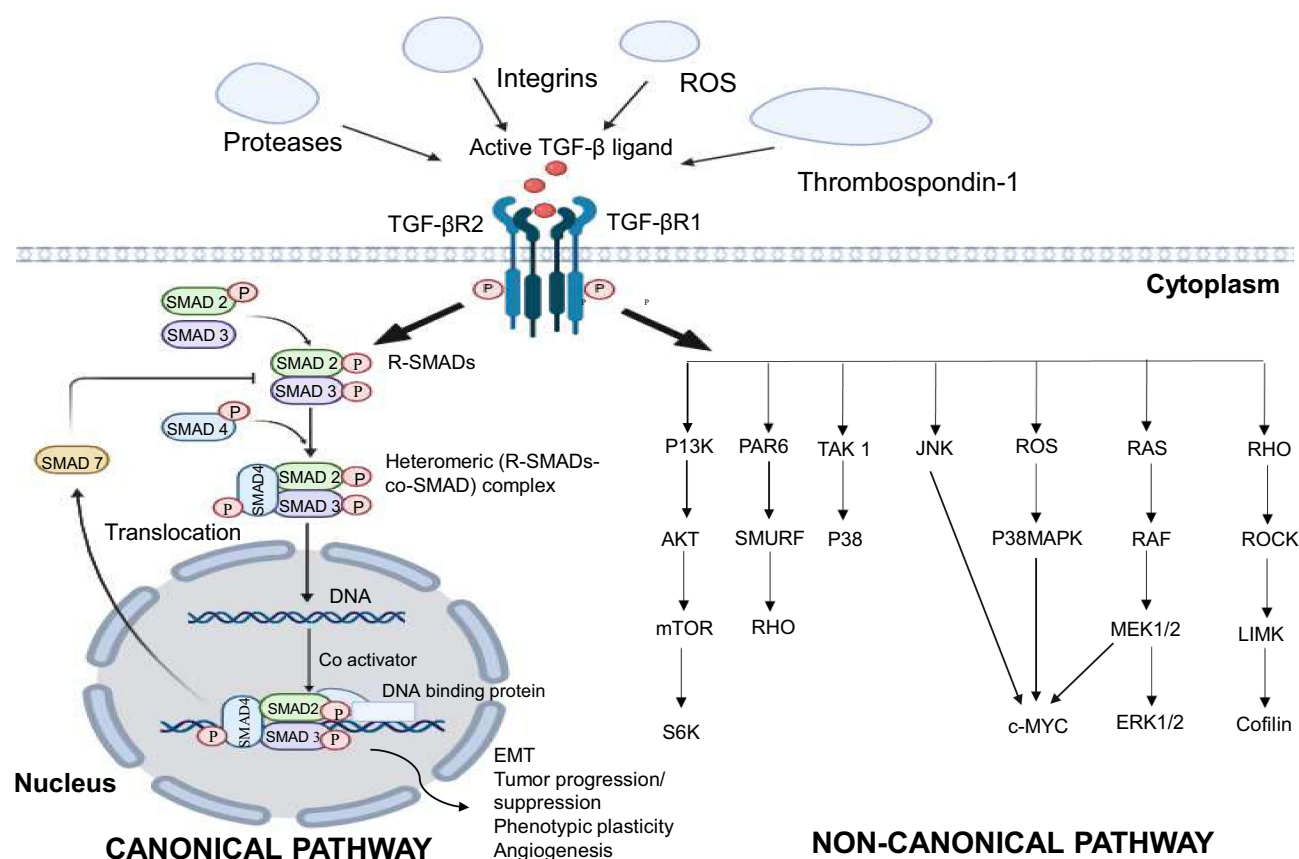


Figure 1 This figure illustrates Canonical (SMAD dependent) and Non-canonical (SMAD independent) TGF-β signaling pathways.^{11,75}

Abbreviations: P13K, Phosphoinositide 3-kinase; mTOR, Mammalian target of rapamycin; S6K, ribosomal protein S6 kinase; PAR6, Partitioning-defective protein 6; TAK1, Transforming growth factor β-activated kinase 1; P, Phosphorus; P38MAPK, Mitogen-activated protein kinases; JNK, C-Jun N-terminal kinase; RAS, Rat sarcoma; RAF, Rapidly accelerated fibrosarcoma; RHO, Rho-associated coiled-coil forming kinase; C-MYC, Myelocytomatosis oncogene cellular homolog; ERK1/2, Extracellular-signal-regulated kinase; LIMK, LIM kinases; EMT, Epithelial–mesenchymal transition; SMAD-R, Receptor-regulated SMADs.

complex.^{78,79} In the case of canonical TGF-β signaling pathways, initially active TGF-β ligands bind with TGF-β receptor type II (TβRII).⁷⁹ It can cause phosphorylation and recruitment in TGF-β receptor type I (TβRI). According to a recent whole exome sequencing (WES) study, TβRII has been observed in 16 most commonly mutated genes in the case of pancreatic cancer.⁸⁰ The TGF-β ligand binding and recruitment trigger TβRII that results in the kinase activation that trans-phosphorylates specific serine/threonine residues of TβRI located in GS domain and intracellular juxta-membrane region consisting of serine and glycine residues.⁸¹ Active TβRI undergoes intracellular signaling with the help of SMADs, proteins transferring signals from TGF-β receptors present on the cell membrane to the nucleus. SMADs have been classified into 3 categories; (i) receptor regulated R-SMADs, (ii) common SMADs, and (iii) inhibitory SMADs. The activated TβRI or activating type I receptors phosphorylates R-SMADs family member 2 (SMAD 2) or 3 (SMAD3) at their two carboxyl-terminal serine residues.⁸² BMP type I receptors, on the other hand, phosphorylate SMAD 1, 5 and 8.⁸³ After phosphorylation, SMAD2/3 dissociates from TβRI and undergo oligomerization of SMAD2 or SMAD3 with SMAD family member 4 (SMAD4), the only known common partner SMAD forming a complex. The heteromeric complex SMAD2/3-SMAD4 results in nuclear translocation⁸⁴ where it is associated with different transcriptional factors regulating the transcriptional repression or activation of target genes highlighted in Table 1.^{85–89}

Canonical signaling carries out the modulation by various mechanism feedback. For instance, TGF-β induces SMAD6 and SMAD7 expression for a negative regulator in TGF-β/SMAD signaling pathway. SMAD7 protein inhibits TGF-β signaling by undergoing various mechanisms, ie, interacting with TβRI and blocking the interactions and phosphorylation between SMAD2/3 and activated TGF-β receptors.¹¹¹ Moreover, SMAD7 also inhibits SMAD2-SMAD4 complex formation and its nuclear translocation^{112,113} along with the interruption in SMAD-DNA complex

Table 1 The Table Illustrates the TGF- β Pathway Target Genes for Tumour Promotion and Suppression

Genes	Function	Tumorigenesis Role	Reference
p15, p21, p57, 4E-BP1, C-MYC	Cell proliferation	Tumor Suppression	[90–92]
E-Cadherin, CK18	EMT suppression	Tumor Suppression	[93,94]
FAS, BAX, BCL-2, PTEN, p53, GADD45B, Granzyme A/B, NKp30, NKG2D	Cell apoptosis	Tumor Suppression	[95–101]
IFN γ , MICA	Immune cells activation	Tumor Suppression	[101,102]
GATA3, T-BET	Inflammation inhibition	Tumor Suppression	[103,104]
FOXP3	Immune suppression	Tumor Promotion	[105]
SNAIL/SLUG, ZEB1/ZEB2, TWIST, VIM, ID1/2/3	EMT activation	Tumor Promotion	[106–110]
HDM2, MMP2, MMP-9, IL11, MUC1, PDGF- β	Metastasis	Tumor Promotion	[111–115]
CTGF, HIF-1 α	Angiogenesis	Tumor Promotion	[116,117]
CDC25A, E2F1	Cell Cycle progression	Tumor Promotion	[118,119]

Abbreviations: CDKN2B, p15: Cyclin-dependent kinase inhibitor 2B; CDKN1A, p21: Cyclin-dependent kinase inhibitor 1; CDKN1C, p57: Cyclin-dependent kinase inhibitor 1C; 4E-BP1, Eukaryotic translation initiation factor 4E-binding protein 1; C-MYC, Myelocytomatosis oncogene cellular homolog; E-Cadherin, Epithelial Cadherin; CK18, Cytokeratin 18; FAS, Fas Cell Surface Death Receptor; BAX, Bcl-2 Associated X Protein; BCL-2, B-Cell Lymphoma 2; PTEN, Phosphatase and Tensin Homolog; p53, Tumor Protein p53; GADD45B, Growth Arrest and DNA Damage Inducible Beta; Granzyme A/B, Granzymes A and B; NKp30, Natural Killer Cell Protein 30; NKG2D, Natural Killer Group 2, Member D; IFN γ , Interferon Gamma; MICA, MHC Class I Polypeptide-Related Sequence A; GATA3, GATA Binding Protein 3; T-BET, T-Box Transcription Factor TBX21; FOXP3, Forkhead Box P3; SNAIL/SLUG, SNAIL/SLUG Transcription Factors; ZEB1/ZEB2, Zinc Finger E-Box Binding Homeobox 1/2; TWIST, Twist Family BHLH Transcription Factor 1; VIM, Vimentin; ID1/2/3, Inhibitor of DNA Binding Proteins 1, 2, and 3; also known as MDM2, Human Double Minute 2 hDM2; MMP2, Matrix Metalloproteinase 2; MMP-9, Matrix Metalloproteinase 9; IL11, Interleukin 11; MUC1, Mucin 1; PDGF- β , Platelet-Derived Growth Factor Subunit B; CTGF, Connective Tissue Growth Factor; HIF-1 α , Hypoxia-Inducible Factor 1-Alpha; CDC25A, Cell Division Cycle 25A; E2F1, E2F Transcription Factor 1.

formation inhibiting TGF- β signalling.¹¹⁴ SMAD ubiquitination regulatory factor 1 (Smurf-1) and E3 ubiquitin ligases also aids in TGF- β signaling regulation due to proteasomal degradation of T β RI.¹¹⁶ Adaptor protein, ie, SMAD anchor for receptor activation (SARA), microtubules and embryonic liver fodrin (ELF) also mediate SMAD's interaction with T β RI necessary for signaling.¹¹⁷

Apart from canonical pathways, TGF- β also activates different intracellular non-canonical (SMAD-independent) signalling pathways in certain type of cells by TGF- β receptor activation.¹¹⁸ In non-canonical signalling pathways, the regulation of actin cytoskeleton changes leading to cell motility, adhesion and growth takes place via Rhodopsin (Rho) like GTPase pathway,¹¹⁹ cell migration and tight junctions via PAR6 regulators,¹²⁰ cell proliferation, survival and metastasis via Extracellular Signal-Regulated Kinases (ERK)/Mitogen Activated Protein Kinases (MAPK) and Phosphatidylinositol-3 Kinase (PI3K)/Akt signaling,^{121–123} cell migration via the RHO/ROCK pathway¹²⁴ and immune evasion, cell survival and inflammation via NF-Kb pathway. These pathways can directly affect the R-SMADs activity. For example, in the ERK signaling pathway, SMAD2/3 is activated via phosphorylation, whereas SMAD3 is sequestered in the cytoplasm for its regulation in the case of the AKT pathway. TGF- β signaling can be activated in many known human cancer types; hence, it is considered an active research topic.

Mucin-1 (MUC1) known as a Type 1 transmembrane glycoprotein, is an oncogene that plays a fundamental role in the modulation of TGF- β signaling in cancer progression and metastasis.^{125–127} MUC1 in normal conditions is restricted to the apical surface of epithelial cells where it serves as a protective barrier.¹²⁸ Meanwhile, in the case of malignant cells, MUC1 does not remain localized to the apical surface; instead, its glycosylation reduces and becomes hypo-glycosylated and causes overexpression of proteins across the cell surface interacting with various growth factor receptors including TGF- β receptors.¹²⁷

In different cancers, tumor-associated MUC1 is overexpressed to enhance EMT, a critical process for cancer metastasis resulting in enhanced drug resistance, metastasis and invasiveness, particularly of EMT-inducing genes.^{125,129–131} As TGF- β induces EMT, MUC1 interacts with TGF- β signaling pathways to regulate its function. Unlike the MUC1 extracellular domain, which acts as a ligand for different receptors, ie, cell adhesion receptors, cytoplasmic tail of MUC1 (MUC1-CT) causes oncogenic signal transduction by undergoing phosphorylation, which

serves in cell invasiveness and metastasis.^{132–134} Once phosphorylated, it gets released from MUC1 N-terminus and binds with β -catenin along with other transcription factors, resulting in the translocation towards nucleus where it undergoes downstream signaling pathways, ie, PI3K/AKT and MAPK pathways.¹³⁵ MUC1-CT is 72 amino acid long, highly conserved domain with seven tyrosine residues phosphorylated by intracellular tyrosine kinases, ie, c-Src, a proto-oncogene molecule having a role in cancer progression.^{125,136,137}

TGF- β /AP-1 Signaling Axis in Cancer Progression

At the core of the shift between TGF- β dual roles, is its interaction with the Activator Protein-1 (AP-1) transcription factor complex, which includes key proteins like c-Fos and c-Jun. This partnership between TGF- β and AP-1 orchestrates numerous downstream effects, driving processes like cell survival, proliferation, invasion, and metastasis. The multifaceted influence of TGF- β and AP-1 on gene expression is critical to the cellular and micro-environmental transformations that define aggressive cancer phenotypes.

TGF- β signals through both canonical (SMAD-dependent) and non-canonical (SMAD-independent) pathways. In the canonical pathway, the translocation of SMAD4 to the nucleus collaborates with AP-1 components like c-Fos and c-Jun, allowing it to regulate genes involved in cellular functions such as growth and differentiation.¹³⁸ This TGF- β /AP-1 collaboration influences genes that modulate ECM production and degradation, promoting invasive behaviors that facilitate cancer cell migration through tissue barriers. In the non-canonical pathway, TGF- β activates AP-1 through other signaling cascades, such as the MAPK, JNK, and PI3K/AKT pathways. For instance, the JNK pathway enhances AP-1 activity by directly phosphorylating c-Jun, which supports gene expression related to stress responses, cellular motility, and invasion.¹³⁹ Meanwhile, the PI3K/AKT pathway promotes apoptosis resistance by stabilizing anti-apoptotic proteins. Through these pathways, AP-1 contributes to cancer cell survival and adaptation, enhancing their resilience to treatments that typically induce cell death.¹⁴⁰

One of the critical roles of TGF- β /AP-1 signaling in cancer is its promotion of epithelial-to-mesenchymal transition (EMT), a process that enables cancer cells to become more migratory and invasive.¹⁴¹ During EMT, cells lose epithelial characteristics like cell–cell adhesion and gain mesenchymal traits such as motility, which are essential for metastatic spread. TGF- β signaling upregulates EMT-related transcription factors (eg, Snail, Slug, and Twist), often in coordination with AP-1.^{142,143} This combined effect represses epithelial markers like E-cadherin and enhances mesenchymal markers such as N-cadherin and vimentin, which reduce cellular adhesion and support migration. Additionally, non-canonical signaling via the JNK pathway enhances AP-1's ability to orchestrate cytoskeletal changes and ECM degradation, facilitating the structural alterations necessary for cancer cell dissemination.¹⁴⁴ In addition, the non-canonical pathways of TGF- β /AP-1 signaling axis are also instrumental in fostering drug resistance. AP-1 can upregulate genes related to drug efflux, DNA repair, and stress response, enabling cancer cells to resist chemotherapy and other targeted treatments.¹⁴⁵

Beyond acting on cancer cells directly, TGF- β and AP-1 modify the tumor microenvironment to favor malignancy. AP-1 regulates ECM-remodeling enzymes, such as matrix metalloproteinases (MMPs), which facilitate tissue breakdown and invasion.^{142,146} This ECM remodeling, enhanced by TGF- β -driven AP-1 activity, enables cancer cells to breach physical barriers, supporting their spread to distant organs. Additionally, AP-1 mediates the expression of pro-inflammatory cytokines like IL-6 and TNF- α , promoting a chronic inflammatory environment that nurtures cancer progression.¹⁴⁷ TGF- β 's activation of AP-1 in this context supports angiogenesis, immune evasion, and additional ECM remodeling, creating a microenvironment conducive to cancer cell survival and adaptation. This inflammatory milieu also supplies cancer cells with growth signals, sustaining tumor expansion and enhancing the resilience of the tumor against therapies. Thus, TGF- β enables AP-1 to coordinate gene expression patterns that promote aggressive cancer traits. This dual influence on cellular and microenvironmental factors highlights the importance of the TGF- β /AP-1 axis as a therapeutic target, especially for slowing tumor growth and enhancing cancer cell susceptibility to treatment.

Cross Talk of TGF- β Signaling Pathway with Other Pathways

TGF- β signaling pathways cross talk with various other intrinsic complex networks, which is a perennial topic in TGF- β study.¹⁴⁸ This cross talk can enhance the understanding of TGF- β role in mediating different biological responses, its

effect on cellular physiology and its role in therapeutics. The cross talk can take place at different levels including ligands, receptor, antagonists and signaling component expression level. These components associate with transcription complexes, induce chromatin modifications, change gene expression and directly interact with SMADs.¹⁴⁹ At early developmental stage, the interactions of TGF- β with BMP, Hedgehog (Hh), Wnt/ Wg, MAPK, Notch and other pathways play a role in cell fate, organogenesis, body configuration and maintenance^{150,151} as highlighted in Figure 2.

Cross Talk with Wnt Signaling Pathway

Wnt signaling pathway involves secreted, lipid-modified signaling molecules responsible for regulating tissue homeostasis, cell fate, migration, survival, self-renewal, and the maintenance of early progenitor and stem cells.¹⁵² Dysregulation in the Wnt pathway is implicated in different cancers, including leukemia and colorectal cancer, where it can lead to aberrant cellular processes.¹⁵³

The canonical Wnt signaling pathway is initiated by Wnt ligands binding to the Frizzled (Fz) receptor along with LRP5/6 co-receptor. This interaction triggers a signaling cascade and is mediated by β -catenin which is a crucial transcriptional co-activator. Upon ligand binding, the intracellular protein Dishevelled (Dvl) becomes activated and inhibits β -catenin destruction complex, which includes Axin, APC (Adenomatous Polyposis Coli), and GSK-3 (Glycogen Synthase Kinase 3). Inhibition of GSK-3 activity prevents the phosphorylation and subsequent degradation of β -catenin, resulting in its accumulation and stabilization in the cytoplasm. Stabilized β -catenin then translocates to nucleus, where it further binds to T-cell factor (TCF) or lymphoid enhancer-binding factor (LEF) transcription factors. This complex recruits co-activators such as CREB-binding protein (CBP) and p300 to drive the expression of Wnt target genes, promoting cell proliferation, differentiation and self-renewal. Hyperactivation of this pathway is a key driver of oncogenesis in various cancers.¹⁵³

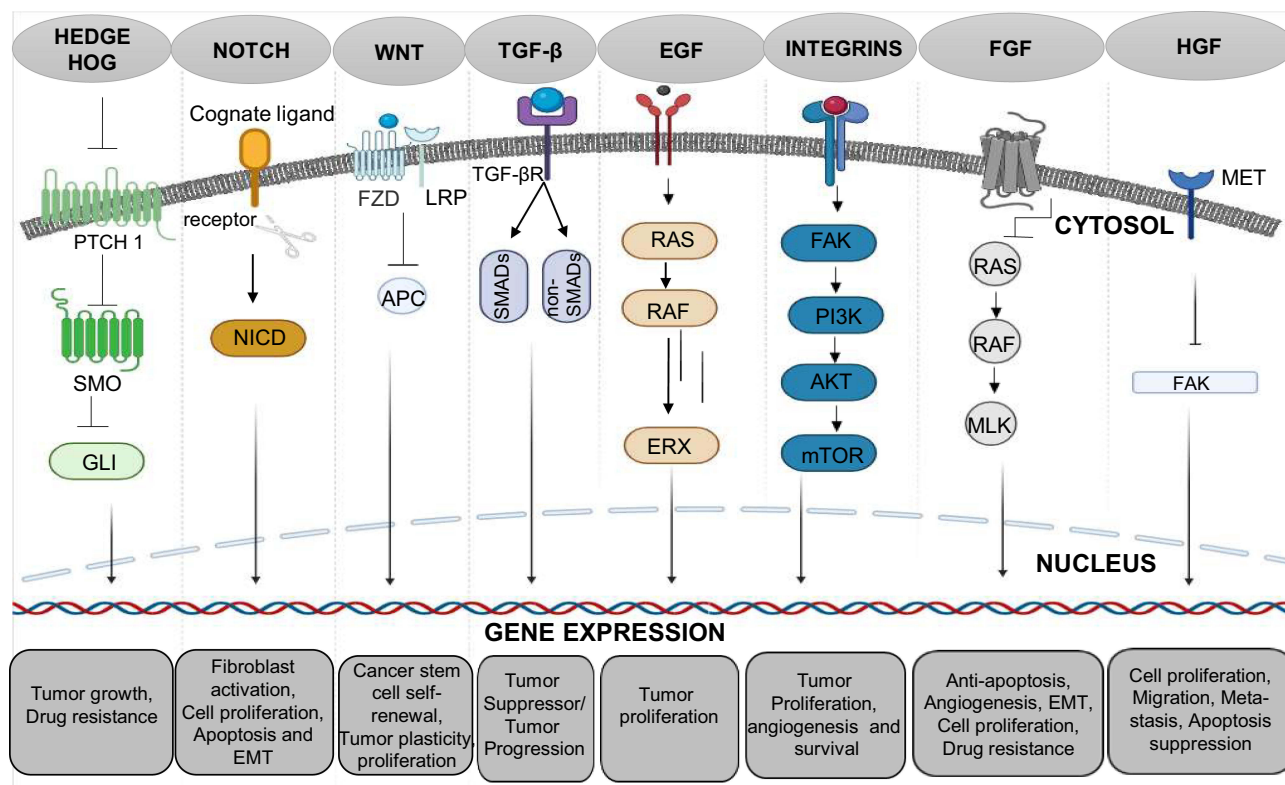


Figure 2 This figure represents the TGF- β signaling pathway cross talk with other related signaling pathways.

Abbreviations: FAK, Focal Adhesion Kinase; NICD, Notch intracellular domain; SMO, Smoothened; FGFs, Fibroblast growth factors; HGF, Hepatocyte growth factor; EGF, Epidermal growth factor; FZDs, Frizzled receptors; LRP, Low-density lipoprotein receptor-related protein; APC, Adenomatous polyposis coli; mTOR, Mammalian target of rapamycin.

The cross talk between TGF- β /BMP and Wnt signaling pathways has been extensively studied, revealing interactions at multiple levels that regulate crucial cellular processes ranging from early development to post-natal tissue homeostasis.^{154,155} First, TGF- β /BMP and Wnt signaling reciprocally regulate their respective ligand production. Second, these pathways interact in the nucleus, where SMAD proteins (mediators of TGF- β signaling pathway) can create complexes with β -catenin and LEF/TCF transcription factors, co-regulating a shared set of target genes and modulating transcriptional activity. Third, cytoplasmic interactions between these pathways are also significant. For example, in *Xenopus*, SMAD4 has been shown to associate with β -catenin in the context of Spemann's organizer, influencing early developmental processes.¹⁵⁶ Additionally, β -catenin signaling is activated by TGF- β via GSK-3 β inactivation, further integrating these pathways.¹²⁵

In the context of cancer, the interaction between TGF- β /Wnt signaling pathways has a pivotal role in promoting metastasis, particularly through their collective influence on epithelial-to-mesenchymal transition (EMT), a process in cancer progression and metastasis. This cross talk not only enhances the invasiveness of cancer cells rather it also contributes in maintaining stem cell-like properties, facilitating tumor spreading and recurrence.

Cross Talk with PI3K/ Akt Signaling Pathway

The PI3K/Akt signaling pathway can show a crucial role in regulating multiple cellular and physiological processes, ie, cell proliferation, invasion, growth, and survival.¹²⁶ Phosphoinositide 3-kinases (PI3Ks) are among the family of lipid kinases and exist in heterodimeric forms and classified into three classes (I, II, and III) based on their (a) structure, (b) distribution, (c) phospholipid substrate specificity, (d) regulatory mechanisms.¹²⁷

PI3K/Akt pathway activation is triggered by various growth factors, cytokines, and cellular stressors through G-protein-coupled receptors (GPCRs) or multiple receptor tyrosine kinases (RTKs). Once it gets activated, PI3K carries out the conversion of PIP2 (phosphatidylinositol 4,5-bisphosphate) into PIP3 (phosphatidylinositol 3,4,5-trisphosphate), a critical second messenger that recruits Akt (also referred as protein kinase B) to the plasma membrane. Akt is then phosphorylated and activated by phosphoinositide-dependent kinase 1 (PDK1) and the mTORC2 complex. Activated Akt regulates numerous downstream targets that are involved in cell survival, metabolism and growth. PI3K/Akt pathway is negatively regulated by the lipid phosphatase PTEN (phosphatase and tensin homolog) dephosphorylating PIP3 back to PIP2, thus acting as a tumor suppressor by inhibiting this pathway.¹²⁸

In many cancers, hyper-activation of PI3K/Akt pathway has been noticed, often due to the mutations or loss of function in PTEN, causing continuous cell growth and survival. The interaction between TGF- β /PI3K/Akt pathway causes additional complexity to cellular regulation. PI3K can be directly or indirectly activated by TGF- β receptors, leading to the activation of PI3K/Akt pathway. This cross talk influences cell fate and self-renewal by upregulating Nanog expression, a key transcription factor responsible for maintaining stem cell pluripotency.¹⁵¹ Moreover, the PI3K/Akt pathway can modulate TGF- β signaling. For instance, Akt can phosphorylate and inhibit SMAD3, TGF- β pathway key mediator, thereby preventing TGF- β -induced apoptosis in hepatocytes.¹²⁹ Akt can also phosphorylate transcription factor FoxO, which undergoes interaction with SMAD3 and inhibits its nuclear translocation, blocking the transcriptional expression of pro-apoptotic genes.¹³⁰ This interaction between TGF- β /PI3K/Akt pathways can promote epithelial-mesenchymal transition (EMT), a critical procedure in cancer progression that enhances cell migration, metastasis and invasion.¹⁴⁹

The cross talk between these pathways also modulates the tumor microenvironment. For example, SMAD-dependent TGF- β signaling can interact with p38 MAPK and PI3K/Akt pathways to activate PFKFB3, an enzyme that drives glycolysis, thus supporting the metabolic demands of rapidly proliferating cancer cells. Conversely, in normal murine mammary gland epithelial cells, the interaction between these pathways can lead to the activation of connexin 43 expression, which is associated with cell-cell communication and homeostasis.^{131,132}

The intricate interaction of TGF- β with PI3K/Akt signaling pathways highlights their combined functions in regulating key cellular processes, including cell survival and differentiation along with cancer progression. This cross talk not only promotes oncogenic processes such as EMT and metastasis but also affects the metabolic adaptation of tumor cells, contributing to their growth and survival in a hostile microenvironment.

Cross Talk with NF- κ B/Rel Signaling Pathway

NF- κ B/Rel signaling pathway is a crucial regulatory network in cellular processes, ie, cell adhesion, senescence, proliferation and survival. NF- κ B/Rel proteins function as dimeric transcription factors and bind to specific DNA sequences present in the nucleus, including the enhancer region of the κ -light chain of the immunoglobulin family. NF- κ B family is classified into two subfamilies; (i) “NF- κ B” proteins (p50/NF- κ B1 and p52/NF- κ B2), (ii) “Rel” proteins (RelA/p65, c-Rel, and RelB).¹³³ Dysregulation in NF- κ B pathway has been linked with various diseases, including arthritis, cancer, cardiovascular diseases, chronic inflammation, asthma, and neurodegenerative disorders.¹³⁴

In the non-canonical NF- κ B pathway, the activation is mediated by a specific group of receptors, such as lymphotoxin- α/β or CD40L receptors. These receptors activate NF- κ B-inducing kinase (NIK), which subsequently phosphorylates IKK α . Phosphorylated IKK α then phosphorylates the carboxy-terminal residues of NF- κ B2 p100, leading to the activation of RelB. NF- κ B2 p100/RelB complex translocates towards the nucleus regulating the expression of a distinct set of genes which is involved in immune responses and cell survival.¹³⁴

The cross talk of TGF- β /NF- κ B pathway is a significant area of study, as these pathways interact in various cellular contexts, particularly in cancer and immune responses. TGF- β activates NF- κ B in a non-canonical manner in various cell types such as head and neck squamous cell carcinoma (HNSCC), osteoblasts, hepatocytes and murine B cells. This activation occurs through TGF- β -activated kinase 1 (TAK1), which is a crucial mediator in this cross talk. Upon TGF- β stimulation, TAK1 is activated which subsequently phosphorylates and activates IKK, resulting in the further activation of NF- κ B. This interaction results in the nuclear translocation of NF- κ B dimers, where they can influence gene expression related to inflammation, cell survival, and proliferation.¹³⁵

In cancer, TGF- β /NF- κ B pathways can have profound implications for tumor progression and metastasis. For instance, in the case of head and neck squamous cell carcinoma (HNSCC), the TGF- β -mediated activation of NF- κ B adds to the aggressive behavior of these tumors by promoting cell survival and apoptosis resistance. Similarly, in the context of chronic inflammation, TGF- β /NF- κ B signaling can cooperate to sustain a pro-inflammatory environment, contributing in the production and progression of cancer and other chronic diseases.

This cross talk highlights the intricate balance of TGF- β /NF- κ B signaling in regulating immune responses and maintaining cellular homeostasis. Dysregulation of this interaction can lead to pathological conditions, including cancer, where the combined activity of these pathways promotes tumor growth, invasion, and therapy resistance. Understanding the molecular mechanisms which underlie this cross talk offers potential therapeutic targets for treating diseases which are relevant to aberrant TGF- β and NF- κ B signaling.

Cross Talk with Hedgehog (Hh) Signaling Pathway

Hedgehog (Hh) signaling pathway is a highly conserved molecular mechanism having a fundamental role in numerous cellular functions, ie, embryonic development and regeneration of tissues. Aberrations occurred in Hh signaling can cause severe developmental defects and tumorigenesis, including the formation of basal cell carcinomas (BCCs) and medulloblastomas.¹³⁶ In mammals, Hh pathway is mediated by three key proteins: (i) Indian Hedgehog (Ihh), (ii) Sonic Hedgehog (Shh), (iii) Desert Hedgehog (Dhh). The initiation takes place by the interaction of Hh ligands with their cell surface receptors; Smoothened (SMO) and Patched (PTCH1 or PTCH2) and controlled intracellularly by Gli (glioma-associated oncogene homolog) factors, specifically Gli 1.2 and 3.¹⁴⁹ When Hh ligands are absent, PTCH 1 and 2 inhibit SMO activity, thereby preventing activation of Gli transcription factors. Upon binding of ligands of Hh to PTCH receptors, the inhibition is relieved, causing SMO to activate Gli, which then migrated to the nucleus regulating target gene expression involved in cell proliferation, survival and differentiation. TGF- β /Hh signaling pathways cross talk occurs on various levels, influencing various functions of cell, particularly during tumorigenesis and embryonic development.¹⁴⁸ TGF- β signaling regulate Hh ligands expression and the activity of Gli transcription factors, thereby modulating the Hh pathway's influence on cell cycle control and differentiation. For example, during embryogenesis, the expression of Shh and other Hh ligands is regulated by TGF- β , influencing the patterning and growth of developing tissues.¹³⁷

One of the key interactions between these pathways involves SMAD3, a major mediator of TGF- β signaling, which interacts with Gli1, enhancing its transcriptional activity. This interaction promotes cell proliferation and survival, which allows to co-regulate cell cycle and differentiation by both TGF- β and Hh pathways. This co-regulation is particularly

evident in developmental processes where TGF- β and Hh signaling cooperate in controlling lineage-specific differentiation and tissue patterning.

In some contexts, Hh/Gli proteins induce the expression of TGF- β signaling components, thereby establishing a feedback loop that further refines cellular responses during development and in disease states such as cancer. SMAD proteins also interact with Gli3, although the functional consequences of this interaction remain to be fully elucidated. However, it is known that in the developing cerebellum, Bone Morphogenetic Proteins (BMP-2 and BMP-4), which are part of TGF- β superfamily, can antagonize the proliferative effects of Sonic Hedgehog (Shh) by downregulating the expression of SMO and Gli1.¹⁵⁷

Additionally, it has been seen that TGF- β inhibits Protein Kinase A (PKA) activity while constantly inducing the expression of Gli1 and Gli2, further modulating the Hh signaling pathway and its downstream effects. This intricate interplay between TGF- β and Hh signaling pathways highlights their combined roles in regulating essential developmental processes and in contributing to pathogenesis of a wide range of diseases, including cancer. Understanding the molecular mechanisms underlying this cross talk offers potential avenues for therapeutic intervention in conditions where these pathways are dysregulated.

Dysregulation of TGF- β Signaling in Cancer

TGF- β is a highly versatile signaling pathway that has a critical part in numerous biological and cellular processes, including the development and regulation of immune system and tissue homeostasis. However, any dysregulation in this pathway can lead to a variety of pathologies, particularly cancer. Different genetic and environmental factors can disrupt TGF- β signaling, leading to impaired cellular functions and contributing to tumorigenesis.^{158,159} One major mechanism of dysregulation in cancer involves genetic along with epigenetic alterations that affect TGF- β receptors, leading to their downregulation or loss of function.¹⁵⁹ This disruption compromises tumor-suppressive effects in TGF- β signaling and facilitates cancer progression. For instance, in certain Mendelian diseases, mutations in TGF- β pathway components result in the impaired development and immune responses, highlighting the essential role of this pathway to maintain normal cellular functions.¹⁶⁰ Gene defects affecting the ligands of TGF- β cytokine family often lead to specific phenotypes, while mutations in downstream signaling components can result in broader and more severe genetic defects. These defects, collectively termed as TGF- β signalopathies, are rare disorders that provide insights into the essential TGF- β signaling functions in the immune system and other biological processes. By understanding that these signalopathies have advanced the development of targeted therapies, it is aimed to correct TGF- β dysregulation with some therapies being safe and effective in clinical studies. In the context of cancer, TGF- β signaling dysregulation allows the tumor cells to evade immune detection and compromise the ability of immune system to fight against the tumor. This immune evasion occurs through two primary mechanisms: (i) immunosuppressive cells induction, ie, regulatory T cells (Tregs), (ii) myeloid-derived suppressor cells (MDSCs), and suppression of immune cell activation. Consequently, the tumor microenvironment can become immunosuppressive, facilitating tumor growth and metastasis.¹⁶¹

The consequences of TGF- β dysregulation in the case of cancer are profound. The pathway, which normally acts as a tumor suppressing pathway by promoting apoptosis and inhibiting cell proliferation, can become pro-tumorigenic when dysregulated. This switch occurs through several mechanisms: First, the loss of tumor-suppressive functions disrupts the ability of TGF- β to activate growth suppressors, leading to unchecked cell proliferation. This alteration often involves changes in both autocrine and paracrine signaling, which inhibit growth-inhibitory effects of TGF- β .¹⁶² Second, during cancer progression, TGF- β signaling pathway may interfere with SMAD proteins, leading to resistance to apoptosis.¹⁶³ This resistance enhances the survival of cancer cell survival, contributing to tumor growth. Third, dysregulation of TGF- β signaling promotes pro-angiogenic factor production, increasing the tumor blood supply and facilitating its growth and metastasis.¹⁶⁴ Fourth, TGF- β dysregulation enhances epithelial-mesenchymal transition (EMT), a process that increases invasiveness in cancer cells and their ability to metastasize to distant organs.^{42,165} Fifth, dysregulation in TGF- β signaling leads to cause defects in DNA repair mechanisms, increasing genomic instability and promoting cancer progression.¹⁶⁶ Sixth, dysregulation in TGF- β signaling contributes in the creation of an inflammatory tumor microenvironment, further promoting tumor growth and survival.¹⁶⁷ Lastly, TGF- β dysregulation can drive metabolic changes in cancer cells, such as altered lipid metabolism, increased glycolysis and enhanced oxidative phosphorylation.¹⁶⁸ These metabolic shifts

provide the energy and biosynthetic precursors necessary for rapid tumor growth. The dysregulation in TGF- β signaling is particularly prevalent in cancers including breast, pancreatic, and colorectal cancer, where it plays a central role in causing metastasis, tumorigenesis and resistance to therapy.¹⁶⁹ Understanding the molecular mechanisms highlighting TGF- β signaling dysregulation in cancer is essential for developing novel therapeutic strategies that aim to restore tumor-suppressive functions of this pathway and combating cancer more effectively.

Dual Role of TGF- β in Cancer Progression

TGF- β has a dual role in cancer progression as shown in Figure 3.¹⁷⁰ It has tumor-suppressive effects during early stages while during advanced stages, it promotes development of tumors in the cells.

Tumor-Suppressive Effects in Early Stages

In the early stages of cancer progression, TGF- β acts as a tumor suppressor, primarily by inhibiting the proliferation of cells and modulating immune responses to maintain cellular homeostasis and prevent the growth and spread of pre-cancerous cells.¹⁷¹ Dysregulation in TGF- β signaling during these initial stages contributes to the initiation and development of various cancers. TGF- β tumor-suppressive effects are mediated through two key transcriptional mechanisms. First, TGF- β induces the cyclin-dependent kinase (CDK) inhibitor expression, ie, p15, p21, and p27, which inhibit CDK activity by blocking cell cycle progression. Second, TGF- β inhibits C-MYC expression, a proto-oncogene promoting the proliferation of cells.¹⁷² By suppressing C-MYC, TGF- β ensures that cells do not proliferate uncontrollably, thus preventing tumor formation. MUC1 transmembrane glycoprotein is also abnormally expressed in many epithelial cancers and impacts various signaling pathways with its upregulation.¹⁷³ In cancer, it involves immune evasion, proliferation, and metastasis. The high levels of MUC1 protein activate TGF- β NF- κ B and β -catenin and

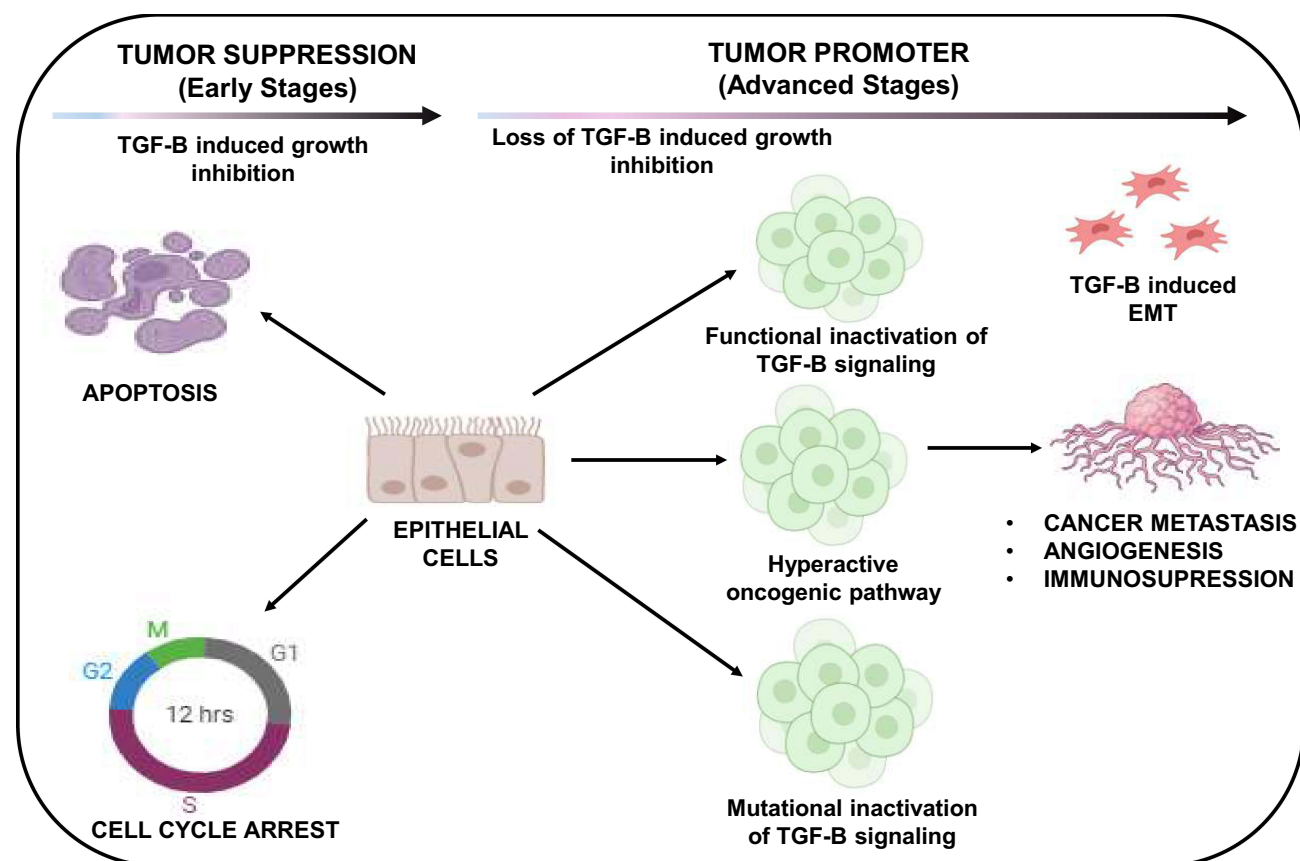


Figure 3 Dual role (early stage; suppression and later stage; promotion) of TGF- β in pancreatic cancer.

Abbreviations: EMT, Epithelial–mesenchymal transition; TGF- β , Transforming Growth Factor Beta.

JNK pathways. This in turn enhances the stability and viability of cells by activating C-MYC in tumor suppressive effects during early stages of development.¹⁷⁴ TGF- β also promotes apoptosis in various cell types as an additional mechanism to prevent tumor growth. This pro-apoptotic effect is mediated through both SMAD-dependent and SMAD-independent pathways.¹⁷⁵ SMAD-dependent pathway causes receptor-regulated SMAD proteins (SMAD2 and SMAD3) phosphorylation and activation, which then results in a complex formation by binding to common-mediator SMAD (SMAD4). This complex migrates towards the nucleus, regulating gene expression involved in apoptosis and cell cycle arrest.¹⁷⁶ The SMAD-independent pathway, although less well understood, is believed to involve alternative signaling molecules and pathways that also contribute to the pro-apoptotic and anti-proliferative effects of TGF- β signaling. The anti-proliferative responses of TGF- β are closely linked to SMAD-dependent pathway, which further underscores its role in tumor suppression. Evidence from various cancer studies supports TGF- β tumor-suppressive role. For instance, in the case of gastric cancer, reduced TGF- β receptors expression has been associated with a loss of growth suppression signaling, highlighting the importance of TGF- β signaling in maintaining normal cellular processes and preventing tumor development.¹⁷⁷ Similarly, in colorectal cancer, the loss of SMAD4, known as a critical mediator of TGF- β signaling, has been linked to reduced growth inhibition, further demonstrating TGF- β tumor-suppressive role in this context.¹⁷⁸ TGF- β signaling has also been implicated in other cancers, including melanoma, hepatocellular carcinoma, and breast cancer, where its dysregulation results in tumor progression. In these cases, the loss of TGF- β 's tumor-suppressive effects may result in the enhancement of cell proliferation, apoptosis, and metastasis, thereby facilitating the transition from early-stage tumors to more aggressive and advanced cancer forms. Understanding the molecular mechanisms underlying TGF- β 's tumor-suppressive functions at early-stage cancers is essential for developing targeted therapies in order to restore its regulatory role and inhibit cancer progression.

Tumor-Promoting Effects in Advanced Stages

During the advanced stages of cancer, TGF- β signaling has a complex and paradoxical role, shifting from a tumor-suppressive function to one that promotes tumor progression and metastasis. This shift acts as a hallmark of various types of cancer, where TGF- β enhances cell invasion, metastasis, and the epithelial–mesenchymal transition (EMT).¹⁷⁹ EMT is a critical process wherein epithelial cells lose their characteristic traits, ie, cell polarity and cell–cell adhesion, and acquire mesenchymal features, including increased migratory and invasive properties. This transition is central to the cancer cells ability to spread and invade other tissues.¹⁸⁰ TGF- β signaling induces EMT via transcriptional regulation of key molecules such as Snail, E-cadherin, vimentin and N-cadherin across various cancers.¹⁸¹ The EMT process involves significant morphological, transcriptional, and translational changes in both epithelial and mesenchymal cells. Normally, epithelial cells are cobblestone-like with polarized structures that maintain tissue integrity. During EMT, these cells' shape and polarity are lost, leading to increased tissue invasion.¹⁸² A hallmark of EMT is the downregulation of E-cadherin, a protein crucial for maintaining cell–cell junctions and structural integrity in epithelial cells. The loss of E-cadherin contributes in the breakdown of epithelial architecture and the transition to a more invasive, mesenchymal phenotype. Several transcription factors are pivotal in orchestrating EMT, including Snail (SNAI1), Slug (SNAI2), Twist1, Twist2, ZEB1, and ZEB2.¹⁸³ Snail and Slug repress E-cadherin expression by binding to E-box regions in its promoter while simultaneously activating mesenchymal genes. Twist proteins further promote EMT by enhancing the expression of mesenchymal markers and suppressing epithelial ones. ZEB factors also play a critical role by repressing E-cadherin and interacting with other transcription factors to reinforce the mesenchymal state.¹⁸⁴ EMT induction by TGF- β can occur through both SMAD-dependent and non-SMAD pathways, leading to cancer progression through a sequence of invasion, circulation, and colonization. The implication of EMT in the progression of several cancers, including breast, lung, liver, and pancreatic cancers, has been seen.¹⁸⁰

Beyond EMT, TGF- β also promotes angiogenesis, immune system evasion, and the creation of an immune-suppressive tumor microenvironment which supports tumor growth and metastasis. As tumors grow, they require an expanded vascular network to supply the necessary nutrients and oxygen for proliferation and metastasis.¹⁸⁵ TGF- β signaling enhances angiogenesis by influencing endothelial cell behavior, increasing their proliferation, migration, and invasion during new blood vessel formation.¹⁸⁶ This angiogenic capability is important for sustaining tumor proliferation and facilitating metastasis. Furthermore, TGF- β plays a role in modulating the immune response, contributing to tumor

immune evasion. It suppresses the activity of dendritic cells, cytotoxic T cells and natural killer (NK) cells, giving rise to a pro-inflammatory environment that diminishes tumor surveillance by host's immune system.^{187,188} This immunosuppressive effect allows tumor cells to evade detection and destruction, further promoting tumor proliferation and metastasis. Thus, at the advanced stages of cancer, TGF- β signaling becomes a powerful promoter of malignancy, contributing to several key processes that drive cancer progression and complicate treatment.

Targeting TGF- β for Cancer Therapy

Cancer treatment remains a significant challenge despite advances in diagnosis and therapeutic options. Issues such as drug resistance and suboptimal response rates persist, necessitating the development of more efficient treatments with minimum side-effects compared to conventional cancer therapies.⁸⁹ One area to focus is therapeutic targeting of specific mediators within TGF- β signaling pathway as they play a dual role in cancer acting as tumour suppressor during early stages but enhancing tumor progression and metastasis later in the advanced stages. Various strategies have been introduced which target these mediators, some of which have shown excellent results in pre-clinical trials in animal models and are currently being evaluated in human clinical trials (as illustrated in Figure 4). Several approaches target various components of TGF- β signaling pathway. These include ligand traps, small molecule inhibitors and monoclonal antibodies which target TGF- β receptors, ligands and downstream signaling molecules like SMAD proteins. For example, small molecule inhibitors (SMIs) such as Galunisertib (LY2157299) specifically inhibit TGF- β type I receptor kinase (ALK5), blocking SMAD2/3 phosphorylation, thereby preventing TGF- β -induced genes transcription associated with tumor progression.¹⁸⁹ Additionally, monoclonal antibodies like fresolimumab (GC1008) can neutralize all three isoforms of TGF- β , reducing its efficiency in promoting immunosuppression, angiogenesis, and metastasis.¹⁹⁰

Another approach involves ligand traps, such as the fusion protein AVID200, which sequesters TGF- β ligands and prevents them from interacting with their receptors.⁶ This method can mitigate the tumour-promoting effects of TGF- β , particularly in those cancers where TGF- β signaling contributes to immune evasion and resistance to therapy. These targeted therapies are not without challenges. One major concern is the potential for unwanted side effects, ie, impaired

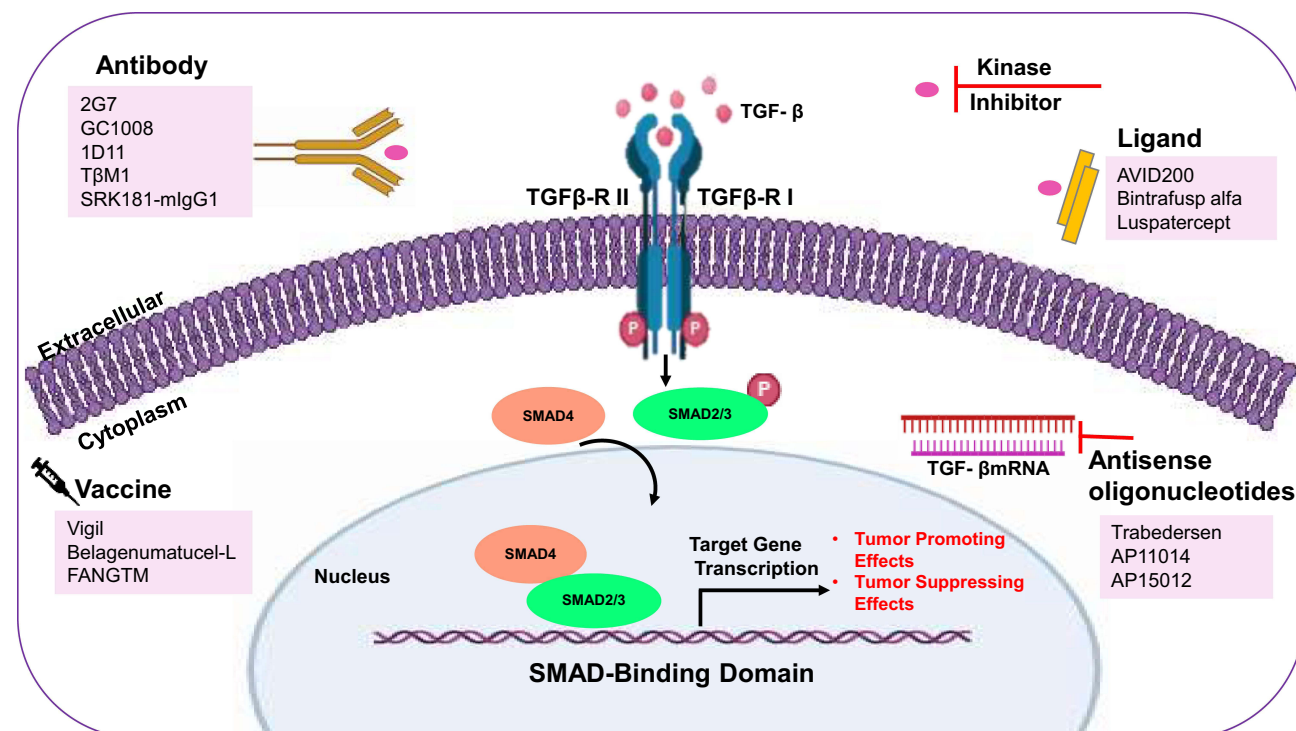


Figure 4 Diagrammatic summary of therapeutic strategies and agents (light purple) targeting the TGF- β signaling pathway. Target gene transcription only indicates the effects regulated by the TGF- β pathway.

Abbreviations: SMAD-R, Receptor-regulated SMADs; P, Phosphorus; SMAD-2, Mothers against decapentaplegic homolog 2.

fibrosis and wound healing due to TGF- β signaling systematic inhibition. Therefore, ongoing clinical trials aim to refine these approaches, improving their efficacy while minimizing adverse effects. The ultimate goal is to develop therapeutic strategies that selectively modulate TGF- β signalling to enhance cancer treatment outcomes without compromising normal physiological functions.

Inhibitors of TGF- β Signaling

TGF- β signaling inhibitor is an important area of research in cancer therapy, aiming to counteract the tumor promoting effects of TGF- β , especially in the case of advanced cancers. Various strategies have become successful in inhibiting TGF- β signaling on various levels, each with unique molecular mechanisms and clinical implications. These include suppressing the TGF- β synthesis, ligands, and its receptor interaction and kinase activity of its receptor.¹⁹¹

At Ligand Level

Antisense oligonucleotides (ASOs) are designed for targeting and degrading TGF- β mRNA, thereby reducing the production of TGF- β proteins.¹⁹² For instance, Trabedersen (AP 12009) which is ASO that targets mRNA of TGF- β II.¹⁹³ It has shown promising results in both pre-clinical trials as well as early-phase clinical trials, particularly when treating high-grade gliomas and pancreatic cancer by downregulating TGF- β 2, leading to reduced tumor growth and improved immune response.^{13,173} However, the stability of ASOs remains a challenge. To address this, nanoparticles and chemical modifications are being employed to enhance their stability and delivery to target different cells and tissues, as demonstrated in recent studies where modified ASOs have shown enhanced tumor-targeting abilities and increased efficacy in reducing TGF- β levels.¹⁷⁴

At Ligand-Receptor Level

Ligand traps and TGF- β neutralizing monoclonal antibodies (mAbs) play role in blocking the interaction between TGF- β ligands and respective receptors, preventing downstream signaling.¹⁹⁴ For example, Fresolimumab (GC1008), a pan-neutralizing TGF- β mAb, has been tested under Phase I/II clinical trials for numerous cancers, ie, renal cell carcinoma and melanoma.¹⁹⁰ These researches indicate that Fresolimumab effectively reduced SMAD2/3 phosphorylation, thereby inhibiting the transcription of pro-tumorigenic genes and reducing tumor progression. Despite the potential, the resistance development and TGF- β signaling complex nature pose challenges in fully harnessing these therapies. In the same context, 1D11, which is a pan-TGF- β neutralizing antibody, usually blocks SMADs phosphorylation resulting in the modulation of TGF- β mediated transfer and spread of breast cancer cells.¹⁹⁵

Intracellular Level

Small molecule inhibitors (SMIs) such as T β RI kinase inhibitor target domain of TGF- β receptors that bind ATP, thereby inhibiting kinase activity as well as downstream signaling.¹⁴² Kinase inhibition causes inhibitory effect of downstream signaling pathways that show reduction in both cancer progression and metastasis in preclinical trials.^{196,197} Galunisertib (LY2157299), a T β RI inhibitor of kinase activity, has been extensively studied under various clinical trials. For instance, in a study of clinical trial Phase II on pancreatic cancer, Galunisertib, combined with standard chemotherapy, showed a significant tumor decrease and improved overall survival, suggesting its potential in combination therapies.¹⁹⁸ However, the risk of off-target and resistance effects are concerns that need to be addressed in ongoing research. Kirin is a T β RI inhibitor Ki26894 that reduces invasiveness and metastasis by inhibitory effects in tumors.¹⁹⁷ SMIs have diverse groups of chemical entities, are easier to produce, are more economical and stable than ASOs and mAbs, and can be administered orally.¹⁹⁹

Vaccine-Based Strategy

Vaccine-based strategy is also under research for targeting TGF- β signaling. For instance, Belagenpumatucel-L, a vaccine comprising irradiated, allogenic lung cancer cells which is genetically modified to block TGF- β II expression, has been tested under clinical trial phase II/III.²⁰⁰ The results indicated that patients receiving this vaccine had improved immune responses and prolonged survival compared to control group, particularly in the case of those patients having less

advanced disease. Another vaccine FANG targets both TGF- β I and II, showing encouraging results in clinical trial phases I/II for advanced solid tumors by restoring immune surveillance and reducing tumor-induced immunosuppression.²⁰¹

Challenges in Targeting TGF- β

Targeting TGF- β signalling in cancer therapy presents both opportunities and significant challenges. While many drugs developed in the past 15 years demonstrated encouraging results when tested under both pre-clinical and clinical trials, several obstacles must be addressed to optimize their effectiveness and minimize adverse effects. One of the primary challenges is managing the adverse effects associated with TGF- β inhibition. TGF- β has a pivotal role in normal cellular mechanisms, ie, regulation of tissue homeostasis, immune system and wound healing. Targeting TGF- β can lead to severe side effects, ie, skin toxicology (eruptive keratoacanthomas, cutaneous squamous, hyperkeratosis, basal cell carcinomas), cardiovascular issues (such as hemorrhagic, inflammatory, and degenerative lesions in heart valves) and immunosuppression.⁵⁵ These side effects compromise the therapeutic potential of TGF- β inhibitors and necessitate careful management. Reducing the drug dosage or optimizing the dosing regimen may help mitigate these adverse effects, but this approach must be balanced with maintaining therapeutic efficacy.

Another challenge is the poor delivery of antisense oligonucleotides (ASOs) to the tumour microenvironment. ASOs are designed to degrade target mRNA and inhibit TGF- β expression, but their effectiveness is limited by poor uptake into tumour cells and rapid degradation by nucleases in the bloodstream.²⁰² To enhance ASO stability and delivery, researchers are exploring the usage of nanoparticles, peptides and liposomes. Despite these advancements, ASOs still face issues such as off-target effects, immune responses, and unpredictable RNA binding affinity. Trabedersen, an ASO targeting TGF- β 2, initially showed promise in early clinical trials but later faced disappointing outcomes due to adverse effects and insufficient targeted delivery.

Monoclonal antibodies (mAbs), another class of TGF- β inhibitors, also present challenges, including limited tissue infiltration, physiological blockage, and structural complexity that limit their uptake by tumor cells. For example, T β M1, a monoclonal antibody targeting TGF- β 1, did not show a promising response to tumor when compared to the inhibitors of pan-TGF- β , possibly due to its low affinity and insufficient tumor penetration.¹⁴² These issues highlight the need for improved mAb design and delivery strategies to enhance their therapeutic potential.

Tumor resistance to TGF- β inhibitors is another significant concern. Tumours can develop resistance through various mechanisms, such as mutations in downstream signalling pathways, which can diminish the effectiveness of TGF- β -targeted therapies. To counteract resistance, combinational therapies that simultaneously target different pathways and identify different biomarkers for patient selection are being explored. Recent research has shown that patients suffering from mesenchymal subtypes of cancer exhibiting high TGF- β target gene expression, have a poorer prognosis, but may benefit more from TGF- β inhibitors.^{203–206} Therefore, transcriptional profiling and biomarker identification are critical for optimizing patient outcomes and improving the effectiveness of TGF- β -targeted therapies.

The heterogeneous nature of cancers further complicates the targeting of TGF- β . Different tumours may exhibit various defects in the TGF- β pathway, leading to a wide range of responses to TGF- β inhibitors among patients. To address this, monitoring TGF- β isoform expression during therapy and using liquid biopsies to track tumour-specific defects is emerging as promising strategies. These approaches may enable more personalized and effective treatments by identifying patients responding more to TGF- β -targeted therapies and allowing for the timely adjustment of therapeutic strategies.

Moreover, due to the complex physiological roles of TGF- β , targeting TGF- β at early disease stages can be detrimental. Due to TGF- β being a tumor suppressor at early stages, targeting TGF- β during this stage can allow tumor progression facilitating the bypass of pre-malignant cells through these regulatory controls. The premature inhibition of TGF- β can alter the tumor-suppressing effect and allow continuous growth and proliferation of tumor.⁵⁸ Similarly, the role of TGF- β in regulating the immune system and preventing autoimmunity, cell differentiation, wound healing and tissue repairing can be affected by its early inhibition leading to hyperactive immune and inflammatory responses, autoimmune diseases, poor wound healing, tissue damage, defects in organogenesis and tissue homeostasis.^{207–209}

In conclusion, while targeting TGF- β signalling holds great potential for cancer therapy, overcoming the associated challenges requires a multifaceted approach. Continued research into optimizing drug delivery, minimizing adverse

effects, understanding resistance mechanisms, and identifying suitable biomarkers is crucial to understanding the therapeutic potential of TGF- β inhibitors completely.

Combination Therapies Under Clinical Trials

A wide range of TGF- β signalling inhibitors (TGF- β antibodies, antisense oligonucleotides (ASOs), and small-molecule inhibitors (SMIs) of T β RI kinase) are currently under clinical trials for the evaluation of their efficacy and safety in many cancer types used either individually or in combination with other anti-cancerous agents, ie, immune checkpoint inhibitors²¹⁰ (Table 2).

TGF-B Inhibitors + Kinase-Targeted Therapies

Several inhibitors have been designed to suppress the kinase activity of TGF-B receptors and pre-clinically tested for cancer treatment. Vactosertib is an oral selective inhibitor molecule that targets the kinase activity of TGF-B. It is designed to be more specific and potent in suppressing tumor proliferation, invasion, and metastasis by blocking TGF- β -mediated signalling.²⁰⁷ It shows great efficacy in limiting growth and inhibits the progression of various solid tumors along with neoplasm multiple myeloma (MM) in murine models.²⁰⁹ Its antitumor activity has been tested in pre-clinical

Table 2 List of Current Anti-TGF β Therapeutic and Combined Strategies in Pre-Clinical Development

Class	Drugs	Target	Type of Cancer	References
Ligand Trap	sBetaglycan PI7, PI44 Cisplatin +/- Bintrafusp alfa Bintrafusp alfa + and PD-LI Brachyury-TRICOM Ado- trastuzumab Emtansine	Pan-TGF- β Pan-TGF- β TGF- β RII and PD-LI TGF- β RII HER2 hDAC deacetylase	Melanoma, renal cell carcinoma, Breast cancer High grade glioma, pancreatic cancer Biliary Tract Cancer treatment, NSCLC Non-small Cell Lung Cancer. Advanced Stage Breast Cancer	[210]
Kinase Inhibitors	LY2109761 A-83-01 TEW-7197 Vactosertib Vactosertib+ Pembrolizumab Vactosertib + Durvalumab Vactosertib+ Pomalidomide Galunisertib+ Vascoterib	T β RII/ALK5 ALK5 T β RI (ALK5) TGF- β TGF- β RI Tubulin TGF- β RI PD-LI TGF- β RI TGF-B, MAPK, PI3K pathways	Several Colon, lung, ovarian Lung, mesothelioma cancer, Combination with FOLFOX in PAC patients Several Solid Tumors, neoplasm multiple myeloma Gastric and colorectal cancer Non-small cell lung cancer, Urothelial cancer Multiple myeloma Hepatocellular carcinoma, solid tumors	[89]
Antisense Oligo-nucleotides	API1014 API2009 (Trabedersen) GC1008 (Fresolimumab) LY2382770 PI44	TGF- β I TGF- β 2 mRNA TGF- β 1, β 2, β 3 T β RI/II complex	NSCLC, CRC, prostate Phase 2 clinical trials Open label Ph I/II in high-grade glioma patients Ph II GC1008 + chemotherapy and radiotherapy in glioma, metastatic breast cancer Ph I trial against prostate carcinoma Monotherapy in skin fibrosis	[89]
Vaccine	Belagenpumatucel-L (Lucanix™) FANG™ or vigil (Gemogenovatucl-T) Vigil + nivolumab Vigil + chemotherapy	TGF- β 2 TGF- β 1, β 2	Lucanix vs placebo in NSCLC, Ph I trial in advanced cancers Systemic therapy in platinum treated NSCLC patients Ovarian Cancer monotherapy, Ewing's sarcoma	[210,211]

(Continued)

Table 2 (Continued).

Class	Drugs	Target	Type of Cancer	References
Monoclonal Antibody	CAT-192 LY238770 GC1008 Fresolimumab+SBRT	TGF- β TGF- β TGF- β TGF- β 1, β 2, β 3 RT	Sclerosis, Hyperkeratosis, Myelofibrosis Trabeculectomy, renal fibrosis Diabetes, Idiopathic Pulmonary Fibrosis, Glomerulosclerosis (FSGS) Stage Ia/Ib NSCLC	[211]

Abbreviations: TGF- β , Transforming Growth Factor Beta; +/-, with or without; NSCLC, Non-small Cell Lung Cancer; RT, Radiation Therapy; ALK5, Activin receptor-like kinase 5; PD-L1, Programmed death-ligand 1; PI3K, Phosphoinositide 3-kinase; MAPK, Mitogen-activated protein kinase.

and clinical trials for various cancer types (advanced solid tumors (NCT02160106) and hematologic malignancies like myelodysplastic syndromes). It is evaluated in Phase 1/2 clinical trials as monotherapy or in combination with other conventional therapies. The study demonstrates that Vactosertib in combination with another therapeutic agent Pembrolizumab (NCT03724851) shows great effectiveness, tolerability, pharmacokinetics, and anti-tumor activity when assessed among patients with gastric and colorectal cancer. In colon cancer patients, 16.7% and 33.3% objective response rates (ORRs) were observed, especially in those patients who do not respond to Pembrolizumab alone.²¹² Vactosertib via tumor intrinsic and extrinsic mechanisms can show pleiotropic effects on multiple cell types.²⁰⁸ Another study claims that its combination with Durvalumab (NCT03732274) has shown 16.7% (ORRs) in patients with non-small cell lung cancer (NSCLC) and urothelial carcinoma, significantly higher than the 2.8% ORR in the same patient group with Durvalumab monotherapy.²¹³ Vactosertib combined with Pomalidomide (NCT03143985) showed safe and progressive-free survival (PFS) of 80% higher than the historical controls with Pomalidomide alone (PFS=20%) or with Pom and corticosteroids (PFS=40%). This phase Ib/IIa study targets relapsed and/or refractory multiple myeloma (MM) with Vactosertib combined with Pomalidomide.²¹⁴

Another TGF-B kinase inhibitor is Galunisertib (LY21557299) developed by Eli Lilly. It has been observed that it blocks the phosphorylation of SMAD2/3 proteins and also inhibits non-canonical pathways (MAPK, PI3K/AKT/mTOR) and prevents the activation of downstream TGF- β signalling simultaneously by trials in HCC cell lines.²¹⁵ It has been evaluated as a mono or combined therapy with other strategies in the 1b/2 phase in clinical trials for pancreatic cancer, glioblastoma (GB), hepatocellular carcinoma (HCC), NSCLC and other solid tumors treatment.²¹⁶ It is one of the most abundantly studied ALK5 inhibitors for clinical trials and has been demonstrated to be a safe and efficient inhibitor having anti-tumoral effects.²⁰⁸ It has shown the potential to reduce the growth of breast and lung cancer cell lines in phase 1 trials. A study has been conducted involving 156 patients given Galunisertib in combination with gemcitabine (GEM) plus placebo GEM in a phase 2 study in patients with pancreatic cancer resulted in an improved OS of 10.9 months compared to 7.2 months in GEM a placebo group as well as progression-free survival (PFS) and toxicity profile compared to placebo plus GEM group.²¹⁷

TGF-B Inhibitors + Immunotherapies

TGF-B shows immunosuppression properties. The inhibition of TGF-B can overcome the immunosuppression of tumor microenvironment. Some immunotherapy strategies have been designed which directly interfere with the TGF-B pathway block its activation and elicit anti-tumor and immune suppressive response.²¹⁸

Lucanix (Belagenpumatucel-L) is a non-viral gene-based allogeneic tumor cell vaccine that inhibits transforming growth factor (TGF-BII). This vaccine reduces the immunosuppressive characteristics of TGF-BII by integrating a TGF-B antisense oligonucleotide-expressing vector into autologous cancer cells.²¹⁹ It has been evaluated in phase 2 clinical trial at different stages for its efficacy in non-small cell lung cancer (NSCLC).²²⁰ The clinical trials showed Belagenpumatucel-L exhibits favorable safety profile and in patients of stages 3B and 4, by giving higher doses of vaccine, there is 2-year overall survival rate.²²¹

SRK181-mIgG1 is a potent selective inhibitor of TGF-B and is designed to precisely target and neutralize the latent TGF-BI complex. The preclinical trial results have shown selective inhibition of TGF-BI including resistance to

checkpoint inhibitors. SRK181-mIgG1 in combination with an immune checkpoint blocked trial showed both safety and effectiveness in mouse cancer models.²²²

Fresolimumab (GC1008, Genzyme), a human monoclonal antibody, broadly neutralizes all three isoforms of TGF- β by interrupting their binding with receptors.²²³ It was assessed during the phase 1 trial with 28 patients diagnosed with advanced renal cell carcinoma (RCC), malignant melanoma, and squamous. During the trials, seven patients showed a partial response. Notable side effects include reversible cutaneous keratoacanthomas and squamous cell carcinomas (SCCs) in four patients and hyperkeratosis in one patient.²²⁴ Fresolimumab which showed the inhibitory effect on TGF- β proving itself safe, tolerable and effective when combined with radiation treatment for cancer patients.²²⁵ The Phase 2 clinical trials have been evaluated in glioma, metastatic breast cancer and relapsed malignant pleural mesothelioma which came out to be greatly tolerant, while evaluation in early-stage non-small cell lung cancer (NSCLC) is still under progress.²²⁶

AVID200 (BMS) is a TGF-B ligand trap. AVID200 neutralizes TGF-BI and TGF-BIII. It does not target TGF-BII, which is a positive regulator of hematopoiesis and normal cardiac function. Since TGF-BI and TGF-BIII are negative regulators of hematopoiesis, while TGF-BI is a positive regulator of hematopoiesis, the unique isoform selectivity profiles of AVID200 make it an attractive agent for the treatment of MDS-associated anaemia. The selective targeting ability of AVID200 makes it an effective and well-tolerated therapeutic in oncology.^{227,228}

Bintrafusp Alfa (GSK-4045154, M7824, MSB0011359C) is a bi-functional fusion protein. It is an innovative approach by combining PD-L1 blockade with TGF- β pathway inhibition, targeting two key mechanisms of immune evasion by tumors. Studies show phase 1 open-label trial in 28 patients with advanced non-small cell lung cancer (NSCLC), and given 500mg or 1200mg of Bintrafusp alfa every two weeks, it showed encouraging efficacy and tolerability in platinum-treated NSCLC patients.²²⁹

AP11014 and AP15012 are antisense oligonucleotide molecules used in pre-clinical trials for the treatment of non-small cell lung cancer, prostate carcinoma, CRC and MM, respectively.²³⁰

AP12009 (Trabedersen, Antisense Pharma GmbH/ Isarna) are ASOs that target TGF-BII expression and are being studied to treat, pancreatic carcinoma and malignant melanoma and glioma with an immunotherapy approach. After pre-clinical trials, the safety and efficacy of AP12009 were evaluated in an open-label phase 1/2 for recurrent and refractory high-grade glioma patients. AP12009 has undergone or is currently used in Phase 3 trials against astrocytoma (NCT00761280).²³¹

FANG is a vaccine created by combining the expression of GM-CSF with that of bi-functional short hairpin RNAi (bi-shRNAi) that targets the furin convertase which has a role in both TGF β I and TGF β II maturation. FANG vaccine has been evaluated in a phase 1 clinical trial demonstrating a good safety profile and immune response induction with prolonged disease control.²³²

TGF-B Inhibitors + Cancer-Stem Cell Therapy

Cancer stem cell (CSC) markers (ALDH high and CD44+/CD24) along with TGF-B are used to treat patients with Triple-negative breast cancer (TNBC). It has shown great enriching effects during chemotherapy of TNBC patients. The CSC markers with the combination of TGF-B inhibitors (paclitaxel) interfere with SMAD4-dependent expressions of IL-8 and inhibit tumorigenesis and the CSC population.²³³

Yadav et al reported that the expression of TGF-BI, II & III enhanced upon treating breast cancer cell lines with radiotherapy along with enhanced migration of CSC markers (CD44+/CD24 & ALD high). However, TGF-BI inhibitors in combination with CSC markers re-sensitized the cells to radiotherapy.²³⁴

Xu et al experimented using epirubicin (widely used anthracycline) for triple-negative breast cancer patients. Through chronic epirubicin exposure, we transformed MDA-MB-231 triple-negative breast cancer cells into epirubicin-resistant cell lines (MB-231/Epi). As a result, the resistant lines showed enhanced TGF-B expression, increased metastatic potential, chemotherapy resistance, and enrichment of CSC markers (CD44+/CD24).²³⁵

Zhu et al study reports that treating TNBC patients with TGF-BI inhibitors increased the expression of mesenchymal markers and decreased the expression of epithelial markers, indicating enhanced migration, metastatic potential and invasion.²³⁶

Another study identified that the use of Ophiopogonin D (an anti-inflammatory agent) disrupts the TGF- β pathway which leads to stimulation of ITGB1/FAK/Src/AKT. In TNBC patients, the TGF- β inhibitors are potent agents to alleviate the pro-metastatic changes stimulated by TGF- β signalling.²³⁶

Other TGF- β Inhibitors

Alternatively, some other compounds have been discovered that show SMAD-dependent transcriptional inhibitory mechanisms. Currently, not much information is available about these compounds. Only two in-vitro pre-clinical studies have been published so far respective to cancer treatment. The first study shows that SiS3 treatment inhibits actin reorganization and migration of PDV-transformed keratinocytes treated with TGF- β 1. The second study shows that the inhibitor was able to block MMP-9 expression induced by either EGF or TGF- β in SKBR3 breast cancer cells, associated with cell migration and invasion. In addition, it has been effectively tested on murine models of skin, pulmonary and hepatic fibrosis by oral administration.^{237,238}

Limitations of TGF- β Antagonists

To use anti-TGF- β therapies in controlling the development of various types of cancer, the dual role of TGF- β needs to be understood better. However, the ubiquitous nature and function of TGF- β (which regulates various physiological processes in cells) limits its mechanistic understanding. Therefore, the limited grasp of the complex dual nature of TGF- β (as a tumor suppressor and tumor promoter) is a challenge in the development of TGF- β antagonists for cancer therapy. Numerous combination therapies and TGF- β pathway inhibitors are being explored, yet the varied effects of TGF- β along with lack of biomarkers, clear patient selection criteria, and optimal dosing protocols still need to be defined. Moreover, several therapeutic agents given alone have shown limited therapeutic activity as compared to combination therapies such as immunotherapy, chemotherapy, and checkpoint inhibitors.

Moreover, patient selection and treatment decisions with the help of molecular biomarkers are beginning to emerge. In the future, the integration of bioinformatics tools and definite biomarkers will prove helpful in identifying patients who would likely respond to TGF- β pathway therapy. Hence, the inclusion of TGF- β receptor antagonists into primary cancer treatment is crucial.²¹⁰

Conclusion

Due to the biphasic role of TGF- β , it has emerged as a potential treatment method for cancer treatment. Although it holds great potential to treat cancer, more research is required to optimize its therapeutic potential. Several combination therapies and TGF- β inhibitors are under clinical trials, providing valuable insights into their safety, efficacy, and possible side effects. Vactosertib, a small potent molecule (kinase receptor inhibitor) in combination with chemotherapy and immunotherapy, seems a promising option to treat various types of cancer. AVID200 led to effective modulation of TGF- β , resulting in immune activation. Bintrafusp alfa (bifunctional fusion protein) has shown encouraging results and manageable side effect profiles in patients. Moreover, combination therapy targeting TGF- β enhances immunotherapy and overcomes resistance by targeting other pathways like (PI3K/AKT or MAPK) along with the TGF- β pathway, reducing metastasis, and also offering tailored treatment based on tumor development and enhancing drug delivery via nanoformulations. In conclusion, TGF- β pathway antagonists, in combination with other potent inhibitor molecules, provide a cost-effective, rapidly acting, and feasible approach to improve cancer treatment. The rational understanding of the TGF- β pathway and its interaction with other immune system processes will help to develop more effective and better strategies for treating cancer.

Data Sharing Statement

The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Acknowledgment

The publication of this article was supported by Qatar University.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. *Carcinogenesis*. 2010;31(1):100–110. doi:10.1093/carcin/bgp263
2. Rajput S, Sharma PK, Malviya R. Fluid mechanics in circulating tumour cells: role in metastasis and treatment strategies. *Med Drug Discov*. 2023;18:100158. doi:10.1016/j.medidd.2023.100158
3. Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov*. 2022;12(1):31–46. doi:10.1158/2159-8290.CD-21-1059
4. Anjum S, Hashim M, Malik SA, et al. Recent advances in zinc oxide nanoparticles (ZnO NPs) for cancer diagnosis, target drug delivery, and treatment. *Cancers*. 2021;13(18):4570. doi:10.3390/cancers13184570
5. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol*. 2012;9(6):703–719. doi:10.4161/rna.20481
6. Shi X, Yang J, Deng S, et al. TGF- β signaling in the tumor metabolic microenvironment and targeted therapies. *J Hematol Oncol*. 2022;15(1):135.
7. Combaran Y, Nguyen TMD. Cell communications among microorganisms, plants, and animals: origin, evolution, and interplays. *Int J Mol Sci*. 2020;21(21):8052. doi:10.3390/ijms21218052
8. Deng Z, Fan T, Xiao C, et al. TGF- β signaling in health, disease, and therapeutics. *Signal Transduction Targeted Ther*. 2024;9(1):61. doi:10.1038/s41392-024-01764-w
9. Poniatowski LA, Wojdasiewicz P, Gasik R, Szukiewicz D. Transforming growth factor Beta family: insight into the role of growth factors in regulation of fracture healing biology and potential clinical applications. *Mediators Inflammation*. 2015;2015(1):137823. doi:10.1155/2015/137823
10. Tzavlaki K, Moustakas A. TGF- β signaling. *Biomolecules*. 2020;10(3):487. doi:10.3390/biom10030487
11. Zhang M, Zhang YY, Chen Y, Wang J, Wang Q, Lu H. TGF- β signaling and resistance to cancer therapy. *Front Cell Develop Biol*. 2021;9:786728. doi:10.3389/fcell.2021.786728
12. Peng D, Fu M, Wang M, Wei Y, Wei X. Targeting TGF- β signal transduction for fibrosis and cancer therapy. *Mol Cancer*. 2022;21(1):104. doi:10.1186/s12943-022-01569-x
13. Zhang Y, Alexander PB, Wang X-F. TGF- β family signaling in the control of cell proliferation and survival. *Cold Spring Harbor Perspect Biol*. 2017;9(4):a022145. doi:10.1101/cshperspect.a022145
14. Dhanasekaran R, Deutzmann A, Mahauad-Fernandez WD, Hansen AS, Gouw AM, Felsher DW. The MYC oncogene—the grand orchestrator of cancer growth and immune evasion. *Nat Rev Clin Oncol*. 2022;19(1):23–36. doi:10.1038/s41571-021-00549-2
15. Wang H-L, Wang L, Zhao C-Y, Lan H-Y. Role of TGF-beta signaling in beta cell proliferation and function in diabetes. *Biomolecules*. 2022;12(3):373. doi:10.3390/biom12030373
16. Xu R, Wu M, Wang Y, et al. Mesenchymal stem cells reversibly de-differentiate myofibroblasts to fibroblast-like cells by inhibiting the TGF- β -SMAD2/3 pathway. *Mol Med*. 2023;29(1):59. doi:10.1186/s10020-023-00630-9
17. Xu X, Zheng L, Yuan Q, et al. Transforming growth factor- β in stem cells and tissue homeostasis. *Bone Res*. 2018;6(1):2. doi:10.1038/s41413-017-0005-4
18. Zhu S, Chen W, Masson A, Li Y-P. Cell signaling and transcriptional regulation of osteoblast lineage commitment, differentiation, bone formation, and homeostasis. *Cell Discov*. 2024;10(1):71.
19. Wang J, Zhao X, Wan YY. Intricacies of TGF- β signaling in treg and Th17 cell biology. *Cell Mol Immunol*. 2023;20(9):1002–1022.
20. Huai G, Markmann JF, Deng S, Rickert CG. TGF- β -secreting regulatory B cells: unsung players in immune regulation. *Clin Transl Immunol*. 2021;10(4):e1270. doi:10.1002/cti2.1270
21. Tan D, Yin W, Guan F, et al. B cell-T cell interplay in immune regulation: a focus on follicular regulatory T and regulatory B cell functions. *Front Cell Develop Biol*. 2022;10:991840. doi:10.3389/fcell.2022.991840
22. Konkel JE, Chen W. Balancing acts: the role of TGF- β in the mucosal immune system. *Trends Mol Med*. 2011;17(11):668–676. doi:10.1016/j.molmed.2011.07.002
23. Foltz JA, Moseman JE, Thakkar A, Chakravarti N, Lee DA. TGF β imprinting during activation promotes natural killer cell cytokine hypersecretion. *Cancers*. 2018;10(11):423. doi:10.3390/cancers10110423
24. Horiguchi M, Ota M, Rifkin DB. Matrix control of transforming growth factor- β function. *J Biochem*. 2012;152(4):321–329. doi:10.1093/jb/mvs089
25. Hu W, Dong A, Karasaki K, et al. Smad4 regulates the nuclear translocation of Nkx2-5 in cardiac differentiation. *Sci Rep*. 2021;11(1):3588. doi:10.1038/s41598-021-82954-2
26. Wang X, Liu T, Huang Y, Dai Y, Lin H. Regulation of transforming growth factor- β signalling by SUMOylation and its role in fibrosis. *Open Biol*. 2021;11(11):210043. doi:10.1098/rsob.210043
27. Zhou P, Wan X, Zou Y, Chen Z, Zhong A. Transforming growth factor beta (TGF- β) is activated by the CtBP2-p300-AP1 transcriptional complex in chronic renal failure. *Int J Bio Sci*. 2020;16(2):204. doi:10.7150/ijbs.38841
28. Deheuninck J, Luo K. Ski and SnoN, potent negative regulators of TGF- β signaling. *Cell Res*. 2009;19(1):47–57. doi:10.1038/cr.2008.324
29. Krstic J, Santibanez JF. Transforming growth factor-beta and matrix metalloproteinases: functional interactions in tumor stroma-infiltrating myeloid cells. *Sci World J*. 2014;2014(1):521754. doi:10.1155/2014/521754
30. Hinz B. The extracellular matrix and transforming growth factor- β 1: tale of a strained relationship. *Matrix Biol*. 2015;47:54–65. doi:10.1016/j.matbio.2015.05.006
31. Tsai C-C, Wu S-B, Kau H-C, Wei Y-H. Essential role of connective tissue growth factor (CTGF) in transforming growth factor- β 1 (TGF- β 1)-induced myofibroblast transdifferentiation from Graves' orbital fibroblasts. *Sci Rep*. 2018;8(1):7276. doi:10.1038/s41598-018-25370-3

32. Ramirez H, Patel SB, Pastar I. The role of TGF β signaling in wound epithelialization. *Adv Wound Care*. 2014;3(7):482–491. doi:10.1089/wound.2013.0466
33. Goumans M-J, Liu Z, Ten Dijke P. TGF- β signaling in vascular biology and dysfunction. *Cell Res*. 2009;19(1):116–127. doi:10.1038/cr.2008.326
34. Li X, Xie R, Luo Y, et al. Cooperation of TGF- β and FGF signalling pathways in skin development. *Cell Proliferation*. 2023;56(11):e13489. doi:10.1111/cpr.13489
35. Liu Z-L, Chen -H-H, Zheng -L-L, Sun L-P, Shi L. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Signal Transduction Targeted Ther*. 2023;8(1):198. doi:10.1038/s41392-023-01460-1
36. Johnson KE, Wilgus TA. Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair. *Adv Wound Care*. 2014;3(10):647–661. doi:10.1089/wound.2013.0517
37. Murphy-Ullrich JE, Suto MJ. Thrombospondin-1 regulation of latent TGF- β activation: a therapeutic target for fibrotic disease. *Matrix Biol*. 2018;68:28–43. doi:10.1016/j.matbio.2017.12.009
38. Grafe I, Alexander S, Peterson JR, et al. TGF- β family signaling in mesenchymal differentiation. *Cold Spring Harbor Perspect Biol*. 2018;10(5):a022202. doi:10.1101/cshperspect.a022202
39. Zinski J, Tajer B, Mullins MC. TGF- β family signaling in early vertebrate development. *Cold Spring Harbor Perspect Biol*. 2018;10(6):a033274. doi:10.1101/cshperspect.a033274
40. Meyers EA, Kessler JA. TGF- β family signaling in neural and neuronal differentiation, development, and function. *Cold Spring Harbor Perspect Biol*. 2017;9(8):a022244. doi:10.1101/cshperspect.a022244
41. Wang P, Rodriguez RT, Wang J, Ghodasara A, Kim SK. Targeting SOX17 in human embryonic stem cells creates unique strategies for isolating and analyzing developing endoderm. *Cell Stem Cell*. 2011;8(3):335–346. doi:10.1016/j.stem.2011.01.017
42. Lee JH, Massagué J, editors. TGF- β in developmental and fibrogenic EMTs. In: *Seminars in Cancer Biology*. Elsevier; 2022.
43. Walton KL, Makani Y, Chen J, et al. Two distinct regions of latency-associated peptide coordinate stability of the latent transforming growth factor- β 1 complex. *J Biol Chem*. 2010;285(22):17029–17037. doi:10.1074/jbc.M110.110288
44. Gentry LE, Lioubin MN, Purchio AF, Marquardt H. Molecular events in the processing of recombinant type 1 pre-pro-transforming growth factor beta to the mature polypeptide. *Mol Cell Biol*. 1988;8(10):4162–4168. doi:10.1128/mcb.8.10.4162-4168.1988
45. Derynck R, Jarrett JA, Chen EY, et al. Human transforming growth factor- β complementary DNA sequence and expression in normal and transformed cells. *Nature*. 1985;316(6030):701–705. doi:10.1038/316701a0
46. Gray AM, Mason AJ. Requirement for activin A and transforming growth factor- β 1 pro-regions in homodimer assembly. *Science*. 1990;247(4948):1328–1330. doi:10.1126/science.2315700
47. Shi M, Zhu J, Wang R, et al. Latent TGF- β structure and activation. *Nature*. 2011;474(7351):343–349. doi:10.1038/nature10152
48. Robertson IB, Horiguchi M, Zilberberg L, Dabovic B, Hadjiolova K, Rifkin DB. Latent TGF- β -binding proteins. *Matrix Biol*. 2015;47:44–53. doi:10.1016/j.matbio.2015.05.005
49. Lyons RM, Keski-Oja J, Moses HL. Proteolytic activation of latent transforming growth factor-beta from fibroblast-conditioned medium. *J Cell Biol*. 1988;106(5):1659–1665. doi:10.1083/jcb.106.5.1659
50. Bonewald L. Transforming growth factor- β . In: *Principles of Bone Biology*. Elsevier; 2002:903–918.
51. Akhurst RJ, Hata A. Targeting the TGF β signalling pathway in disease. *Nat Rev Drug Discov*. 2012;11(10):790–811. doi:10.1038/nrd3810
52. Barcellos-Hoff M, Dix TA. Redox-mediated activation of latent transforming growth factor-beta 1. *Mol Endocrinol*. 1996;10(9):1077–1083. doi:10.1210/mend.10.9.8885242
53. Jobling MF, Mott JD, Finnegan MT, et al. Isoform-specific activation of latent transforming growth factor β (LTGF- β) by reactive oxygen species. *Radiat Res*. 2006;166(6):839–848. doi:10.1667/RR0695.1
54. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGF β activation. *J Cell Sci*. 2003;116(2):217–224. doi:10.1242/jcs.00229
55. Batlle E, Massagué J. Transforming growth factor- β signaling in immunity and cancer. *Immunity*. 2019;50(4):924–940. doi:10.1016/j.immuni.2019.03.024
56. Lyons RM, Gentry LE, Purchio A, Moses HL. Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. *J Cell Biol*. 1990;110(4):1361–1367. doi:10.1083/jcb.110.4.1361
57. Qin Y, Garrison BS, Ma W, et al. A milieu molecule for TGF- β required for microglia function in the nervous system. *Cell*. 2018;174(1):156–71.e16. doi:10.1016/j.cell.2018.05.027
58. Kobayashi T, Kim H, Liu X, et al. Matrix metalloproteinase-9 activates TGF- β and stimulates fibroblast contraction of collagen gels. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(11):L1006–L115. doi:10.1152/ajplung.00015.2014
59. Saharinen J, Taipale J, Keski-Oja J. Association of the small latent transforming growth factor-beta with an eight cysteine repeat of its binding protein LTBP-1. *EMBO J*. 1996;15(2):245–253. doi:10.1002/j.1460-2075.1996.tb00355.x
60. Miyazono K, Olofsson A, Colosetti P, Heldin C-H. A role of the latent TGF-beta 1-binding protein in the assembly and secretion of TGF-beta 1. *EMBO J*. 1991;10(5):1091–1101. doi:10.1002/j.1460-2075.1991.tb08049.x
61. Taipale J, Miyazono K, Heldin C-H, Keski-Oja J. Latent transforming growth factor-beta 1 associates to fibroblast extracellular matrix via latent TGF-beta binding protein. *J Cell Biol*. 1994;124(1):171–181. doi:10.1083/jcb.124.1.171
62. Zilberberg L, Todorovic V, Dabovic B, et al. Specificity of latent TGF- β binding protein (LTBP) incorporation into matrix: role of fibrillins and fibronectin. *J Cell Physiol*. 2012;227(12):3828–3836. doi:10.1002/jcp.24094
63. García-Sainz JA, Vilchis-Landeros MM, Juárez P, López-Casillas F, Hernández-Pando R, Massagué J. Receptores y funciones del TGF-beta, una citocina crucial en la cicatrización [Receptors and functions of TGF-beta, a crucial cytokine in wound healing]. *Gaceta Médica de México*. Spanish. 2003;139(2):126–143.
64. Liénart S, Merceron R, Vanderaa C, et al. Structural basis of latent TGF- β 1 presentation and activation by GARP on human regulatory T cells. *Science*. 2018;362(6417):952–956. doi:10.1126/science.aau2909
65. Tran DQ, Andersson J, Wang R, Ramsey H, Unutmaz D, Shevach EM. GARP (LRRC32) is essential for the surface expression of latent TGF- β on platelets and activated FOXP3+ regulatory T cells. *Proc Natl Acad Sci*. 2009;106(32):13445–13450. doi:10.1073/pnas.0901944106
66. Bachmann M, Kukkurainen S, Hytönen VP, Wehrle-Haller B. Cell adhesion by integrins. *Physiol Rev*. 2019;99(4):1655–1699. doi:10.1152/physrev.00036.2018

67. Lodyga M, Hinz B, editors. TGF- β 1—a truly transforming growth factor in fibrosis and immunity. In: *Seminars in Cell & Developmental Biology*. Elsevier; 2020.
68. Morris DG, Huang X, Kaminski N, et al. Loss of integrin α v β 6-mediated TGF- β activation causes Mmp12-dependent emphysema. *Nature*. 2003;422(6928):169–173. doi:10.1038/nature01413
69. Humphries M. Monoclonal antibodies as probes of integrin priming and activation. *Biochem Soc Trans*. 2004;32(3):407–411. doi:10.1042/bst0320407
70. Worthington JJ, Klementowicz JE, Travis MA. TGF β : a sleeping giant awoken by integrins. *Trends Biochem Sci*. 2011;36(1):47–54. doi:10.1016/j.tibs.2010.08.002
71. Hyttiäinen M, Penttinen C, Keski-Oja J. Latent TGF- β binding proteins: extracellular matrix association and roles in TGF- β activation. *Crit Rev Clin Lab Sci*. 2004;41(3):233–264. doi:10.1080/10408360490460933
72. Rifkin DB. Latent transforming growth factor- β (TGF- β) binding proteins: orchestrators of TGF- β availability. *J Biol Chem*. 2005;280(9):7409–7412. doi:10.1074/jbc.R400029200
73. Wakefield LM, Winokur TS, Hollands RS, Christopherson K, Levinson AD, Sporn MB. Recombinant latent transforming growth factor beta 1 has a longer plasma half-life in rats than active transforming growth factor beta 1, and a different tissue distribution. *J Clin Invest*. 1990;86(6):1976–1984. doi:10.1172/JCI114932
74. Derynck R, Zhang Y, Feng X-H. Transcriptional activators of TGF- β responses: smads. *Cell*. 1998;95(6):737–740. doi:10.1016/S0092-8674(00)81696-7
75. Costanza B, Umelo IA, Bellier J, Castronovo V, Turtoi A. Stromal modulators of TGF- β in cancer. *J Clin Med*. 2017;6(1):7. doi:10.3390/jcm6010007
76. Yamashita H, ten Dijke P, Franzen P, Miyazono K, Heldin C-H. Formation of hetero-oligomeric complexes of type I and type II receptors for transforming growth factor-beta. *J Biol Chem*. 1994;269(31):20172–20178. doi:10.1016/S0021-9258(17)32142-7
77. Weis-Garcia F, Massague J. Complementation between kinase-defective and activation-defective TGF-beta receptors reveals a novel form of receptor cooperativity essential for signaling. *EMBO J*. 1996;15(2):276–289. doi:10.1002/j.1460-2075.1996.tb00358.x
78. Bilandzic M, Stenvers KL. Betaglycan: a multifunctional accessory. *Mol Cell Endocrinol*. 2011;339(1–2):180–189. doi:10.1016/j.mce.2011.04.014
79. Franzen P, ten Dijke P, Ichijo H, et al. Cloning of a TGF β type I receptor that forms a heteromeric complex with the TGF β type II receptor. *Cell*. 1993;75(4):681–692. doi:10.1016/0092-8674(93)90489-D
80. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature*. 2012;491(7424):399–405. doi:10.1038/nature11547
81. Wrana JL, Attisano L, Wieser R, Ventura F, Massagué J. Mechanism of activation of the TGF- β receptor. *Nature*. 1994;370(6488):341–347. doi:10.1038/370341a0
82. Souchelnytskyi S, Tamaki K, Engström U, Wernstedt C, Ten Dijke P, Heldin C-H. Phosphorylation of Ser465 and Ser467 in the C terminus of Smad2 mediates interaction with Smad4 and is required for transforming growth factor- β signaling. *J Biol Chem*. 1997;272(44):28107–28115. doi:10.1074/jbc.272.44.28107
83. Miyazawa K, Shinozaki M, Hara T, Furuya T, Miyazono K. Two major Smad pathways in TGF- β superfamily signalling. *Genes Cells*. 2002;7(12):1191–1204. doi:10.1046/j.1365-2443.2002.00599.x
84. Heldin C-H, Miyazono K, Ten Dijke P. TGF- β signalling from cell membrane to nucleus through SMAD proteins. *Nature*. 1997;390(6659):465–471. doi:10.1038/37284
85. Heldin C-H, Moustakas A. Signaling receptors for TGF- β family members. *Cold Spring Harbor Perspect Biol*. 2016;8(8):a022053. doi:10.1101/cshperspect.a022053
86. Derynck R, Budi EH. Specificity, versatility, and control of TGF- β family signaling. *Sci Signaling*. 2019;12(570):eaav5183. doi:10.1126/scisignal.aav5183
87. Nishihara A, Hanai J, Okamoto N, et al. Role of p300, a transcriptional coactivator, in signalling of TGF- β . *Genes Cells*. 1998;3(9):613–623. doi:10.1046/j.1365-2443.1998.00217.x
88. Liu S, Ren J, Ten Dijke P. Targeting TGF β signal transduction for cancer therapy. *Signal Transduction Targeted Ther*. 2021;6(1):8. doi:10.1038/s41392-020-00436-9
89. Kim B-G, Malek E, Choi SH, Ignatz-Hoover JJ, Driscoll JJ. Novel therapies emerging in oncology to target the TGF- β pathway. *J Hematol Oncol*. 2021;14:1–20. doi:10.1186/s13045-021-01053-x
90. Katz LH, Li Y, Chen J-S, et al. Targeting TGF- β signaling in cancer. *Expert Opin Ther Targets*. 2013;17(7):743–760. doi:10.1517/14728222.2013.782287
91. Massagué J. TGF β in cancer. *Cell*. 2008;134(2):215–230. doi:10.1016/j.cell.2008.07.001
92. Petroulakis E, Mamane Y, Le Bacquer O, Shahbazian D, Sonenberg N. mTOR signaling: implications for cancer and anticancer therapy. *Br J Cancer*. 2006;94(2):195–199. doi:10.1038/sj.bjc.6602902
93. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial–mesenchymal transitions. *Nat Rev Mol Cell Biol*. 2006;7(2):131–142. doi:10.1038/nrm1835
94. Miettinen PJ, Ebner R, Lopez AR, Derynck R. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. *J Cell Biol*. 1994;127(6):2021–2036. doi:10.1083/jcb.127.6.2021
95. Kirkin V, Joos S, Zörnig M. The role of Bcl-2 family members in tumorigenesis. *Biochim Biophys Acta Mol Cell Res*. 2004;1644(2–3):229–249. doi:10.1016/j.bbamcr.2003.08.009
96. Vousden KH, Prives C. Blinded by the light: the growing complexity of p53. *Cell*. 2009;137(3):413–431. doi:10.1016/j.cell.2009.04.037
97. Adams JM, Cory S. Bcl-2-regulated apoptosis: mechanism and therapeutic potential. *Curr Opin Immunol*. 2007;19(5):488–496. doi:10.1016/j.coi.2007.05.004
98. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase–AKT pathway in human cancer. *Nat Rev Cancer*. 2002;2(7):489–501. doi:10.1038/nrc839
99. Li D, Dai C, Yang X, Li B, Xiao X, Tang S. GADD45a regulates olaquinox-induced DNA damage and S-phase arrest in human hepatoma G2 cells via JNK/p38 pathways. *Molecules*. 2017;22(1):124. doi:10.3390/molecules22010124

100. Chowdhury D, Lieberman J. Death by a thousand cuts: granzyme pathways of programmed cell death. *Annu Rev Immunol.* **2008**;26(1):389–420. doi:10.1146/annurev.immunol.26.021607.090404
101. Castriconi R, Cantoni C, Della Chiesa M, et al. Transforming growth factor β 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. *Proc Natl Acad Sci.* **2003**;100(7):4120–4125. doi:10.1073/pnas.0730640100
102. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. *Nat Rev Immunol.* **2006**;6(11):836–848. doi:10.1038/nri1961
103. Kourou-Mehr H, Bechis SK, Slorach EM, et al. GATA-3 links tumor differentiation and dissemination in a luminal breast cancer model. *Cancer Cell.* **2008**;13(2):141–152. doi:10.1016/j.ccr.2008.01.011
104. Chi X, Luo S, Ye P, et al. T-cell exhaustion and stemness in antitumor immunity: characteristics, mechanisms, and implications. *Front Immunol.* **2023**;14:1104771. doi:10.3389/fimmu.2023.1104771
105. Feuerer M, Hill JA, Mathis D, Benoist C. Foxp3⁺ regulatory T cells: differentiation, specification, subphenotypes. *Nat Immunol.* **2009**;10(7):689–695. doi:10.1038/ni.1760
106. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer.* **2007**;7(6):415–428. doi:10.1038/nrc2131
107. Gheldof A, Berx G. Cadherins and epithelial-to-mesenchymal transition. *Prog mol Biol Transl Sci.* **2013**;116:317–336.
108. Yang J, Mani SA, Donaher JL, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell.* **2004**;117(7):927–939. doi:10.1016/j.cell.2004.06.006
109. Mendez MG, Kojima SI, Goldman RD. Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. *FASEB J.* **2010**;24(6):1838–1851. doi:10.1096/fj.09-151639
110. Lasorella A, Benezra R, Iavarone A. The ID proteins: master regulators of cancer stem cells and tumour aggressiveness. *Nat Rev Cancer.* **2014**;14(2):77–91. doi:10.1038/nrc3638
111. Putoczki TL, Ernst M. IL-11 signaling as a therapeutic target for cancer. *Immunotherapy.* **2015**;7(4):441–453. doi:10.2217/imt.15.17
112. Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev.* **2006**;25:9–34. doi:10.1007/s10555-006-7886-9
113. Heldin C-H, Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev.* **1999**;79:1283–1316. doi:10.1152/physrev.1999.79.4.1283
114. Kung C-P, Weber JD. It's getting complicated—a fresh look at p53-MDM2-ARF triangle in tumorigenesis and cancer therapy. *Front Cell Develop Biol.* **2022**;10:818744. doi:10.3389/fcell.2022.818744
115. Stamenkovic I, editor. Matrix metalloproteinases in tumor invasion and metastasis. In: *Seminars in Cancer Biology*. Elsevier; **2000**.
116. Tenney RCV. *Effect of Hypoxia in Skeletal Muscle Fibrosis: Regulation of CTGF/CCN2 Expression by HIF-1 α and TGF- β* . Chile: Pontificia Universidad Catolica de Chile; **2019**.
117. Zhong H, De Marzo AM, Laughner E, et al. Overexpression of hypoxia-inducible factor 1 α in common human cancers and their metastases. *Cancer Res.* **1999**;59(22):5830–5835.
118. Boutros R, Lobjois V, Ducommun B. CDC25 phosphatases in cancer cells: key players? Good targets? *Nat Rev Cancer.* **2007**;7(7):495–507. doi:10.1038/nrc2169
119. Johnson DG, DeGregori J. Putting the oncogenic and tumor suppressive activities of E2F into context. *Curr Mol Med.* **2006**;6(7):731–738. doi:10.2174/1566524010606070731
120. Miyazawa K, Miyazono K. Regulation of TGF- β family signaling by inhibitory Smads. *Cold Spring Harbor Perspect Biol.* **2017**;9(3):a022095. doi:10.1101/cshperspect.a022095
121. Nakao A, Afrakhte M, Morn A, et al. Identification of Smad7, a TGF β -inducible antagonist of TGF- β signalling. *Nature.* **1997**;389(6651):631–635. doi:10.1038/39369
122. Hayashi H, Abdollah S, Qiu Y, et al. The MAD-related protein Smad7 associates with the TGF β receptor and functions as an antagonist of TGF β signaling. *Cell.* **1997**;89(7):1165–1173. doi:10.1016/S0092-8674(00)80303-7
123. Shi X, Chen F, Yu J, et al. Study of interaction between Smad7 and DNA by single-molecule force spectroscopy. *Biochem Biophys Res Commun.* **2008**;377(4):1284–1287. doi:10.1016/j.bbrc.2008.10.145
124. Ebisawa T, Fukuchi M, Murakami G, et al. Smurf1 interacts with transforming growth factor- β type I receptor through Smad7 and induces receptor degradation. *J Biol Chem.* **2001**;276(16):12477–12480. doi:10.1074/jbc.C100008200
125. Apostolopoulos V, Pietersz GA, Tsibani A, et al. Pilot Phase III immunotherapy study in early-stage breast cancer patients using oxidized mannan-MUC1 [ISRCTN71711835]. *Breast Cancer Res.* **2006**;8:1–11. doi:10.1186/bcr1505
126. Kufe DW. Functional targeting of the MUC1 oncogene in human cancers. *Cancer Biol Ther.* **2009**;8(13):1197–1203. doi:10.4161/cbt.8.13.8844
127. Kato K, Lillehoj EP, Lu W, Kim KC. MUC1: the first respiratory mucin with an anti-inflammatory function. *J Clin Med.* **2017**;6(12):110. doi:10.3390/jcm6120110
128. Kaur S, Kumar S, Momi N, Sasson AR, Batra SK. Mucins in pancreatic cancer and its microenvironment. *Nat Rev Gastroenterol Hepatol.* **2013**;10(10):607–620. doi:10.1038/nrgastro.2013.120
129. Zhou R, Curry JM, Roy LD, et al. A novel association of neuropilin-1 and MUC1 in pancreatic ductal adenocarcinoma: role in induction of VEGF signaling and angiogenesis. *Oncogene.* **2016**;35(43):5608–5618. doi:10.1038/onc.2015.516
130. Roy LD, Sahraei M, Subramani DB, et al. MUC1 enhances invasiveness of pancreatic cancer cells by inducing epithelial to mesenchymal transition. *Oncogene.* **2011**;30(12):1449–1459. doi:10.1038/onc.2010.526
131. Singh PK, Hollingsworth MA. Cell surface-associated mucins in signal transduction. *Trends Cell Biol.* **2006**;16(9):467–476. doi:10.1016/j.tcb.2006.07.006
132. Thompson EJ, Shanmugam K, Kotlarczyk KL, et al. Tyrosines in the MUC1 cytoplasmic tail modulate oncogenic signaling pathways. *Cancer Res.* **2005**;65(9_Supplement):222.
133. Li Q, Liu G, Shao D, et al. Mucin1 mediates autocrine transforming growth factor beta signaling through activating the c-Jun N-terminal kinase/activator protein 1 pathway in human hepatocellular carcinoma cells. *Int J Biochem Cell Biol.* **2015**;59:116–125. doi:10.1016/j.biocel.2014.11.012

134. Li Y, Kuwahara H, Ren J, Wen G, Kufe D. The c-Src tyrosine kinase regulates signaling of the human DF3/MUC1 carcinoma-associated antigen with GSK3 β and β -catenin. *J Biol Chem*. 2001;276(9):6061–6064. doi:10.1074/jbc.C000754200
135. Li Q, Kuwahara H, Yin L, et al. The epidermal growth factor receptor regulates interaction of the human DF3/MUC1 carcinoma antigen with c-Src and β -catenin. *J Biol Chem*. 2001;276(38):35239–35242. doi:10.1074/jbc.C100359200
136. Ren J, Raina D, Chen W, Li G, Huang L, Kufe D. MUC1 oncoprotein functions in activation of fibroblast growth factor receptor signaling. *mol Cancer Res*. 2006;4(11):873–883. doi:10.1158/1541-7786.MCR-06-0204
137. Guo X, Wang X-F. Signaling cross-talk between TGF- β /BMP and other pathways. *Cell Res*. 2009;19(1):71–88. doi:10.1038/cr.2008.302
138. Sundqvist A, Vasilaki E, Voytyuk O, et al. TGF β and EGF signaling orchestrates the AP-1-and p63 transcriptional regulation of breast cancer invasiveness. *Oncogene*. 2020;39(22):4436–4449. doi:10.1038/s41388-020-1299-z
139. Sundqvist A, Voytyuk O, Hamdi M, et al. JNK-dependent cJun phosphorylation mitigates TGF β -and EGF-induced pre-malignant breast cancer cell invasion by suppressing AP-1-mediated transcriptional responses. *Cells*. 2019;8(12):1481. doi:10.3390/cells8121481
140. Zhang L, Zhou F, ten Dijke P. Signaling interplay between transforming growth factor- β receptor and PI3K/AKT pathways in cancer. *Trends Biochem Sci*. 2013;38(12):612–620. doi:10.1016/j.tibs.2013.10.001
141. Kang Q, Peng X, Li X, et al. Calcium channel protein ORAI1 mediates TGF- β induced epithelial-to-mesenchymal transition in colorectal cancer cells. *Front Oncol*. 2021;11:649476. doi:10.3389/fonc.2021.649476
142. Hao Y, Baker D, Ten Dijke P. TGF- β -mediated epithelial-mesenchymal transition and cancer metastasis. *Int J Mol Sci*. 2019;20(11):2767. doi:10.3390/ijms20112767
143. Ibrahim WN, Doolaanea AA, Bin Abdull Rasad MSB. Effect of shRNA mediated silencing of YB-1 protein on the expression of matrix collagenases in malignant melanoma cell in vitro. *Cells*. 2018;7(1):7. doi:10.3390/cells7010007
144. Tam SY, Law HK. JNK in tumor microenvironment: present findings and challenges in clinical translation. *Cancers*. 2021;13(9):2196. doi:10.3390/cancers13092196
145. Song D, Lian Y, Zhang L. The potential of activator protein 1 (AP-1) in cancer targeted therapy. *Front Immunol*. 2023;14:1224892. doi:10.3389/fimmu.2023.1224892
146. Wisam N, Ridhwan A, Syaiful A. Expression of collagenases matrix metalloproteinases and YB-1 oncogenic factor in malignant melanoma cancer cells and its regulation by stromal fibroblasts. *Int J Cancer Res*. 2017;13:17–25.
147. Deichaite I, Sears TJ, Sutton L, et al. Differential regulation of TNF α and IL-6 expression contributes to immune evasion in prostate cancer. *J Transl Med*. 2022;20(1):527. doi:10.1186/s12967-022-03731-x
148. Tang W, Ling G, Sun L, Liu F-Y. Smad anchor for receptor activation (SARA) in TGF-beta signaling. *Front Biosci*. 2010;2:857–860.
149. Zhang YE. Non-smad signaling pathways of the TGF- β family. *Cold Spring Harbor Perspect Biol*. 2017;9(2):a022129. doi:10.1101/cshperspect.a022129
150. Vardouli L, Moustakas A, Stournaras C. LIM-kinase 2 and cofilin phosphorylation mediate actin cytoskeleton reorganization induced by transforming growth factor- β . *J Biol Chem*. 2005;280(12):11448–11457. doi:10.1074/jbc.M402651200
151. Ozdamar B, Bose R, Barrios-Rodiles M, Wang H-R, Zhang Y, Wrana JL. Regulation of the polarity protein Par6 by TGF β receptors controls epithelial cell plasticity. *Science*. 2005;307(5715):1603–1609. doi:10.1126/science.1105718
152. Lee MK, Pardoux C, Hall MC, et al. TGF- β activates Erk MAP kinase signalling through direct phosphorylation of ShcA. *EMBO J*. 2007;26(17):3957–3967. doi:10.1038/sj.emboj.7601818
153. Yamashita M, Fatyol K, Jin C, Wang X, Liu Z, Zhang YE. TRAF6 mediates Smad-independent activation of JNK and p38 by TGF- β . *Molecular Cell*. 2008;31(6):918–924. doi:10.1016/j.molcel.2008.09.002
154. Chen R-H, Su Y-H, Chuang RL, Chang T-Y. Suppression of transforming growth factor- β -induced apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway. *Oncogene*. 1998;17(15):1959–1968. doi:10.1038/sj.onc.1202111
155. Macias MJ, Martin-Malpartida P, Massagué J. Structural determinants of Smad function in TGF- β signaling. *Trends Biochem Sci*. 2015;40(6):296–308. doi:10.1016/j.tibs.2015.03.012
156. Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends Mol Med*. 2014;20(6):332–342. doi:10.1016/j.molmed.2014.02.007
157. Luo K. Signaling cross talk between TGF- β /Smad and other signaling pathways. *Cold Spring Harbor Perspect Biol*. 2017;9(1):a022137. doi:10.1101/cshperspect.a022137
158. Wu M, Chen G, Li Y-P. TGF- β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. *Bone Res*. 2016;4(1):1–21.
159. Gordeeva O. TGF β family signaling pathways in pluripotent and teratocarcinoma stem cells' fate decisions: balancing between self-renewal, differentiation, and cancer. *Cells*. 2019;8(12):1500. doi:10.3390/cells8121500
160. Huelsken J, Birchmeier W. New aspects of Wnt signaling pathways in higher vertebrates. *Curr Opin Genet Dev*. 2001;11(5):547–553. doi:10.1016/S0959-437X(00)00231-8
161. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev*. 1997;11(24):3286–3305. doi:10.1101/gad.11.24.3286
162. Nusse R. Wnt signaling in disease and in development. *Cell Res*. 2005;15(1):28–32. doi:10.1038/sj.cr.7290260
163. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004;20:781–810. doi:10.1146/annurev.cellbio.20.010403.113126
164. Nishita M, Hashimoto MK, Ogata S, et al. Interaction between Wnt and TGF- β signalling pathways during formation of Spemann's organizer. *Nature*. 2000;403(6771):781–785. doi:10.1038/35001602
165. Clifford RL, Deacon K, Knox AJ. Novel regulation of vascular endothelial growth factor-A (VEGF-A) by transforming growth factor β 1: requirement for Smads, β -catenin, and GSK3 β . *J Biol Chem*. 2008;283(51):35337–35353. doi:10.1074/jbc.M803342200
166. Rodgers SJ, Ferguson DT, Mitchell CA, Ooms LM. Regulation of PI3K effector signalling in cancer by the phosphoinositide phosphatases. *Biosci Rep*. 2017;37(1):BSR20160432. doi:10.1042/BSR20160432
167. Piddock R, Loughran N, Marlein C, et al. PI3K δ and PI3K γ isoforms have distinct functions in regulating pro-tumoural signalling in the multiple myeloma microenvironment. *Blood Cancer J*. 2017;7(3):e539–e. doi:10.1038/bcj.2017.16

168. Haddadi N, Lin Y, Travis G, Simpson AM, Nassif NT, McGowan EM. PTEN/PTENP1: 'Regulating the regulator of RTK-dependent PI3K/Akt signalling', new targets for cancer therapy. *Mol Cancer*. 2018;17:1–14. doi:10.1186/s12943-018-0803-3
169. Papoutsoglou P, Louis C, Coulouarn C. Transforming growth factor-beta (TGFβ) signaling pathway in cholangiocarcinoma. *Cells*. 2019;8(9):960. doi:10.3390/cells8090960
170. Yadav RK, Chauhan AS, Zhuang L, Gan B, editors. FoxO transcription factors in cancer metabolism. In: *Seminars in Cancer Biology*. Elsevier; 2018.
171. Rodríguez-García A, Samsó P, Fontova P, et al. TGF-β1 targets Smad, p38 MAPK, and PI 3K/Akt signaling pathways to induce PFKFB 3 gene expression and glycolysis in glioblastoma cells. *FEBS J*. 2017;284(20):3437–3454. doi:10.1111/febs.14201
172. Tacheau C, Fontaine J, Loy J, Mauviel A, Verrecchia F. TGF-β induces connexin43 gene expression in normal murine mammary gland epithelial cells via activation of p38 and PI3K/AKT signaling pathways. *J Cell Physiol*. 2008;217(3):759–768. doi:10.1002/jcp.21551
173. Yang Y, Ye W-L, Zhang R-N, et al. The role of TGF-β signaling pathways in cancer and its potential as a therapeutic target. *Evid Based Complement Alternat Med*. 2021;2021(1):6675208. doi:10.1155/2021/6675208
174. Zi Z, Chapnick DA, Liu X. Dynamics of TGF-β/Smad signaling. *FEBS Lett*. 2012;586(14):1921–1928. doi:10.1016/j.febslet.2012.03.063
175. Tilborghs S, Corthouts J, Verhoeven Y, et al. The role of nuclear factor-kappa B signaling in human cervical cancer. *Crit Rev Oncol Hematol*. 2017;120:141–150. doi:10.1016/j.critrevonc.2017.11.001
176. Hinz M, Scheidereit C. The IκB kinase complex in NF-κB regulation and beyond. *EMBO Rep*. 2014;15(1):46–61. doi:10.1002/embr.201337983
177. Arsuru M, Panta GR, Bilyeu JD, et al. Transient activation of NF-κB through a TAK1/IKK kinase pathway by TGF-β1 inhibits AP-1/SMAD signaling and apoptosis: implications in liver tumor formation. *Oncogene*. 2003;22(3):412–425. doi:10.1038/sj.onc.1206132
178. Hooper JE, Scott MP. Communicating with hedgehogs. *Nat Rev Mol Cell Biol*. 2005;6(4):306–317. doi:10.1038/nrm1622
179. Perrot CY, Javelaud D, Mauviel A. Overlapping activities of TGF-β and Hedgehog signaling in cancer: therapeutic targets for cancer treatment. *Pharmacol Ther*. 2013;137(2):183–199. doi:10.1016/j.pharmthera.2012.10.002
180. Rios I, Alvarez-Rodríguez R, Martí E, Pons S. Bmp2 antagonizes sonic hedgehog-mediated proliferation of cerebellar granule neurones through Smad5 signalling; 2004.
181. Massagué J, Sheppard D. TGF-β signaling in health and disease. *Cell*. 2023;186(19):4007–4037. doi:10.1016/j.cell.2023.07.036
182. Chowdhury S, Ammanamanchi S, Howell GM. Epigenetic targeting of transforming growth factor β receptor II and implications for cancer therapy. *Mol Cell Pharm*. 2009;1(1):57. doi:10.4255/mcpharmacol.09.07
183. Rodari MM, Cerf-Bensussan N, Parlato M. Dysregulation of the immune response in TGF-β signalopathies. *Front Immunol*. 2022;13:1066375. doi:10.3389/fimmu.2022.1066375
184. van den Bulk J, de Miranda NF, Ten Dijke P. Therapeutic targeting of TGF-β in cancer: hacking a master switch of immune suppression. *Clin Sci*. 2021;135(1):35–52. doi:10.1042/CS20201236
185. Xue VW, Chung JY-F, Córdoba CAG, et al. Transforming growth factor-β: a multifunctional regulator of cancer immunity. *Cancers*. 2020;12(11):3099. doi:10.3390/cancers12113099
186. Wang Q, Xiong F, Wu G, et al. SMAD proteins in TGF-β signalling pathway in cancer: regulatory mechanisms and clinical applications. *Diagnostics*. 2023;13(17):2769. doi:10.3390/diagnostics13172769
187. Chan M-K-K, Chan EL-Y, Ji ZZ, et al. Transforming growth factor-β signaling: from tumor microenvironment to anticancer therapy. *Explor Targeted Anti Tumor Ther*. 2023;4(2):316. doi:10.37349/etat.2023.00137
188. Kuburich NA, Sabapathy T, Demestichas BR, Maddela JJ, den Hollander P, Mani SA, editors. Proactive and reactive roles of TGF-β in EMT-induced plasticity. In: *Seminars in Cancer Biology*. Elsevier; 2023.
189. Liu Q, Lopez K, Murnane J, Humphrey T, Barcellos-Hoff MH. Misrepair in context: tGFβ regulation of DNA repair. *Front Oncol*. 2019;9:799. doi:10.3389/fonc.2019.00799
190. Liu S, Chen S, Zeng J. TGF-β signaling: a complex role in tumorigenesis. *Mol Med Rep*. 2018;17(1):699–704. doi:10.3892/mmr.2017.7970
191. Principe DR, Doll JA, Bauer J, et al. TGF-β: duality of function between tumor prevention and carcinogenesis. *J Natl Cancer Inst*. 2014;106(2):djt369. doi:10.1093/jnci/djt369
192. Tie Y, Tang F, Peng D, Zhang Y, Shi H. TGF-beta signal transduction: biology, function and therapy for diseases. *Mol Biomed*. 2022;3(1):45. doi:10.1186/s43556-022-00109-9
193. Seoane J, Gomis RR. TGF-β family signaling in tumor suppression and cancer progression. *Cold Spring Harbor Perspect Biol*. 2017;9(12):a022277. doi:10.1101/cshperspect.a022277
194. Baba AB, Rah B, Bhat GR, et al. Transforming growth factor-beta (TGF-β) signaling in cancer-A betrayal within. *Front Pharmacol*. 2022;13:791272. doi:10.3389/fphar.2022.791272
195. Itatani Y, Kawada K, Sakai Y. Transforming growth factor-β signaling pathway in colorectal cancer and its tumor microenvironment. *Int J mol Sci*. 2019;20(23):5822. doi:10.3390/ijms20235822
196. Ribatti D, Tamma R, Annese T. Epithelial-mesenchymal transition in cancer: a historical overview. *Transl Oncol*. 2020;13(6):100773. doi:10.1016/j.tranon.2020.100773
197. Moustakas A, Heldin C-H. Mechanisms of TGFβ-induced epithelial–mesenchymal transition. *J Clin Med*. 2016;5(7):63. doi:10.3390/jcm5070063
198. Kim DH, Xing T, Yang Z, Dudek R, Lu Q, Chen Y-H. Epithelial mesenchymal transition in embryonic development, tissue repair and cancer: a comprehensive overview. *J Clin Med*. 2017;7(1):1. doi:10.3390/jcm7010001
199. Radhakrishnan K, Truong L, Carmichael CL. An “unexpected” role for EMT transcription factors in hematological development and malignancy. *Front Immunol*. 2023;14:1207360. doi:10.3389/fimmu.2023.1207360
200. Serrano-Gomez SJ, Maziveyi M, Alahari SK. Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. *mol Cancer*. 2016;15:1–14. doi:10.1186/s12943-016-0502-x
201. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell mol Life Sci*. 2020;77:1745–1770. doi:10.1007/s00018-019-03351-7
202. Sanjabi S, Oh SA, Li MO. Regulation of the immune response by TGF-β: from conception to autoimmunity and infection. *Cold Spring Harbor Perspect Biol*. 2017;9(6):a022236. doi:10.1101/cshperspect.a022236

203. Herbertz S, Sawyer JS, Stauber AJ, et al. Clinical development of galunisertib (LY2157299 monohydrate), a small molecule inhibitor of transforming growth factor-beta signaling pathway. *Drug Des Devel Ther.* **2015**;9:4479–4499. doi:10.2147/DDDT.S86621
204. Morris JC, Tan AR, Olencki TE, et al. Phase I study of GC1008 (fresolimumab): a human anti-transforming growth factor-beta (TGFβ) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma. *PLoS One.* **2014**;9(3):e90353. doi:10.1371/journal.pone.0090353
205. Varricchio L, Iancu-Rubin C, Upadhyaya B, et al. TGF-β1 protein trap AVID200 beneficially affects hematopoiesis and bone marrow fibrosis in myelofibrosis. *JCI Insight.* **2021**;6(18). doi:10.1172/jci.insight.145651
206. Sabbadini F, Bertolini M, De Matteis S, et al. The multifaceted role of TGF-β in gastrointestinal tumors. *Cancers.* **2021**;13(16):3960. doi:10.3390/cancers13163960
207. Jin CH, Krishnaiah M, Sreenu D, et al. Discovery of N-((4-([1, 2, 4] Triazolo [1, 5-a] Pyridin-6-Yl)-5-(6-Methylpyridin-2-Yl)-1 h-Imidazol-2-Yl) methyl)-2-Fluoroaniline (EW-7197): a highly potent, selective, and orally bioavailable inhibitor of TGF-β type I receptor kinase as cancer immunotherapeutic/antifibrotic agent. *J Med Chem.* **2014**;57(10):4213–4238. doi:10.1021/jm500115w
208. Son JY, Park S-Y, Kim S-J, et al. EW-7197, a novel ALK-5 kinase inhibitor, potently inhibits breast to lung metastasis. *Mol Cancer Ther.* **2014**;13(7):1704–1716. doi:10.1158/1535-7163.MCT-13-0903
209. Jung SY, Hwang S, Clarke JM, et al. Pharmacokinetic characteristics of vactosertib, a new activin receptor-like kinase 5 inhibitor, in patients with advanced solid tumors in a first-in-human phase 1 study. *Invest New Drugs.* **2020**;38:812–820. doi:10.1007/s10637-019-00835-y
210. Huang C-Y, Chung C-L, Hu T-H, Chen -J-J, Liu P-F, Chen C-L. Recent progress in TGF-β inhibitors for cancer therapy. *Biomed Pharmacother.* **2021**;134:111046. doi:10.1016/j.biopha.2020.111046
211. Fabregat I, Fernando J, Mainez J, Sancho P. TGF-beta signaling in cancer treatment. *Curr Pharm Des.* **2014**;20(17):2934–2947. doi:10.2174/13816128113199990591
212. Lee K-W, Park YS, Ahn JB, et al. Safety and anti-tumor activity of the transforming growth factor beta receptor I kinase inhibitor, vactosertib, in combination with pembrolizumab in patients with metastatic colorectal or gastric cancer. *J ImmunoTher Cancer.* **2019**;7:1.
213. Jiang M, Peng W, Pu X, et al. Peripheral blood biomarkers associated with outcome in non-small cell lung cancer patients treated with nivolumab and durvalumab monotherapy. *Front Oncol.* **2020**;10:913. doi:10.3389/fonc.2020.00913
214. Tsukada T, Fushida S, Harada S, et al. Low-dose paclitaxel modulates tumour fibrosis in gastric cancer. *Int J Oncol.* **2013**;42(4):1167–1174. doi:10.3892/ijo.2013.1801
215. Dituri F, Mazzocca A, Peidrò FJ, et al. Differential inhibition of the TGF-β signaling pathway in HCC cells using the small molecule inhibitor LY2157299 and the D10 monoclonal antibody against TGF-β receptor type II. *PLoS One.* **2013**;8(6):e67109. doi:10.1371/journal.pone.0067109
216. Serova M, Tijeras-Raballand A, Dos Santos C, et al. Effects of TGF-beta signaling inhibition with LY2157299 in hepatocarcinoma models and in ex vivo whole tumor tissue samples from patient specimen. *Cancer Res.* **2013**;73(8_Supplement):2094. doi:10.1158/1538-7445.AM2013-2094
217. Fujiwara Y, Nokihara H, Yamada Y, et al. Phase 1 study of galunisertib, a TGF-beta receptor I kinase inhibitor, in Japanese patients with advanced solid tumors. *Cancer Chemother Pharmacol.* **2015**;76:1143–1152. doi:10.1007/s00280-015-2895-4
218. Arteaga C, Hurd S, Winnier A, Johnson M, Fendly B, Forbes J. Anti-transforming growth factor (TGF)-beta antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity. Implications for a possible role of tumor cell/host TGF-beta interactions in human breast cancer progression. *J Clin Invest.* **1993**;92(6):2569–2576. doi:10.1172/JCI116871
219. Giaccone G, Bazhenova L, Nemunaitis J, et al. A phase III study of belagenpumatucel-L, an allogeneic tumour cell vaccine, as maintenance therapy for non-small cell lung cancer. *Eur J Cancer.* **2015**;51(16):2321–2329. doi:10.1016/j.ejca.2015.07.035
220. Nemunaitis J, Dillman RO, Schwarzenberger PO, et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J Clin Oncol.* **2006**;24(29):4721–4730. doi:10.1200/JCO.2005.05.5335
221. Neuzillet C, Tijeras-Raballand A, Cohen R, et al. Targeting the TGFβ pathway for cancer therapy. *Pharmacol Ther.* **2015**;147:22–31. doi:10.1016/j.pharmthera.2014.11.001
222. Martin CJ, Datta A, Littlefield C, et al. Selective inhibition of TGFβ1 activation overcomes primary resistance to checkpoint blockade therapy by altering tumor immune landscape. *Sci Trans Med.* **2020**;12(536):eaay8456. doi:10.1126/scitranslmed.aay8456
223. Vincenti F, Fervenza FC, Campbell KN, et al. A phase 2, double-blind, placebo-controlled, randomized study of fresolimumab in patients with steroid-resistant primary focal segmental glomerulosclerosis. *Kidney Int Rep.* **2017**;2(5):800–810. doi:10.1016/j.ekir.2017.03.011
224. Lacouture ME, Morris JC, Lawrence DP, et al. Cutaneous keratoacanthomas/squamous cell carcinomas associated with neutralization of transforming growth factor β by the monoclonal antibody fresolimumab (GC1008). *Cancer Immunol Immunother.* **2015**;64:437–446. doi:10.1007/s00262-015-1653-0
225. Gonzalez-Junca A, Reiners O, Borrero-Garcia LD, et al. Positron emission tomography imaging of functional TGFβ activity and benefit of TGFβ Inhibition in irradiated intracranial tumors. *Int J Radiat Oncol Biol Phys.* **2020**;109(2):527. doi:10.1016/j.ijrobp.2020.09.043
226. Stevenson JP, Kindler HL, Papasavvas E, et al. Immunological effects of the TGFβ-blocking antibody GC1008 in malignant pleural mesothelioma patients. *Oncoimmunology.* **2013**;2(8):e26218. doi:10.4161/onci.26218
227. O'Connor-McCourt MD, Tremblay G, Lenferink A, Sulea T, Zwaagstra J, Koropatnick J. AVID200, a highly potent TGF-beta trap, exhibits optimal isoform selectivity for enhancing anti-tumor T-cell activity, without promoting metastasis or cardiotoxicity. *Cancer Res.* **2018**;78(13_Supplement):1759. doi:10.1158/1538-7445.AM2018-1759
228. Pfeiffer N, Voykov B, Renieri G, et al. First-in-human phase I study of ISTH0036, an antisense oligonucleotide selectively targeting transforming growth factor beta 2 (TGF-β2), in subjects with open-angle glaucoma undergoing glaucoma filtration surgery. *PLoS One.* **2017**;12(11):e0188899. doi:10.1371/journal.pone.0188899
229. Strauss J, Heery CR, Schlom J, et al. Phase I trial of M7824 (MSB0011359C), a bifunctional fusion protein targeting PD-L1 and TGFβ, in advanced solid tumors. *Clin Cancer Res.* **2018**;24(6):1287–1295. doi:10.1158/1078-0432.CCR-17-2653
230. Hau P, Jachimczak P, Schlingensiepen R, et al. Inhibition of TGF-β 2 with ap 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. *Oligonucleotides.* **2007**;17(2):201–212. doi:10.1089/oli.2006.0053
231. Lampropoulos P, Zizi-Sermpetzoglou A, Rizos S, Kostakis A, Nikiteas N, Papavassiliou AG. TGF-beta signalling in colon carcinogenesis. *Cancer Lett.* **2012**;314(1):1–7. doi:10.1016/j.canlet.2011.09.041

232. Schlingensiepen K-H, Schlingensiepen R, Steinbrecher A, et al. Targeted tumor therapy with the TGF- β 2 antisense compound AP 12009. *Cytokine Growth Factor Rev.* **2006**;17(1–2):129–139. doi:10.1016/j.cytogfr.2005.09.002
233. Bhola NE, Balko JM, Dugger TC, et al. TGF- β inhibition enhances chemotherapy action against triple-negative breast cancer. *J Clin Invest.* **2013**;123(3):1348–1358. doi:10.1172/JCI65416
234. Yadav P, Shankar BS. Radio resistance in breast cancer cells is mediated through TGF- β signalling, hybrid epithelial-mesenchymal phenotype and cancer stem cells. *Biomed Pharmacother.* **2019**;111:119–130. doi:10.1016/j.biopha.2018.12.055
235. Xu X, Zhang L, He X, et al. TGF- β plays a vital role in triple-negative breast cancer (TNBC) drug-resistance through regulating stemness, EMT and apoptosis. *Biochem Biophys Res Commun.* **2018**;502(1):160–165. doi:10.1016/j.bbrc.2018.05.139
236. Zhu X, Wang K, Chen Y. Ophiopogonin D suppresses TGF- β 1-mediated metastatic behavior of MDA-MB-231 breast carcinoma cells via regulating ITGB1/FAK/Src/AKT/ β -catenin/MMP-9 signaling axis. *Toxicol In Vitro.* **2020**;69:104973. doi:10.1016/j.tiv.2020.104973
237. Jinnin M, Ihn H, Tamaki K. Characterization of SIS3, a novel specific inhibitor of Smad3, and its effect on transforming growth factor- β 1-induced extracellular matrix expression. *Mol Pharmacol.* **2006**;69(2):597–607. doi:10.1124/mol.105.017483
238. Kocic J, Bugarski D, Santibanez JF. SMAD3 is essential for transforming growth factor- β 1-induced urokinase type plasminogen activator expression and migration in transformed keratinocytes. *Eur J Cancer.* **2012**;48(10):1550–1557. doi:10.1016/j.ejca.2011.06.043

OncoTargets and Therapy

Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/oncotargets-and-therapy-journal>

Dovepress

Taylor & Francis Group