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REVIEW

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

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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-κB, 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,

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sustained proliferative signaling, stimulation of angiogenesis, evasion of growth suppressors, permitting replicative immorality, 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-βI, TGF-βII, and TGF-βIII.[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 cytostasis 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-βRII), which cause activation and phosphorylation of TGF-β type I receptor (TGF-βRI). When TGF-βRI 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 nuclease.[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 αvβ3 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, goosecoid 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 bond[46] and form a small latent complex (SLC) by binding non-covalently with LAP. A proteolytic cleavage side 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 (αvβ6, αvβ8) 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-βI, II, and III. TGF-βI 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 TβRIs (ALK 1) and 2 TβRIIs.[76,77] Betaglycan is the third type of TGF-β receptor (TβRIII) which is a low affinity, non-signaling, co-receptor abundant on different cell surfaces binding TGF-β ligands to high-affinity TGF-β receptor

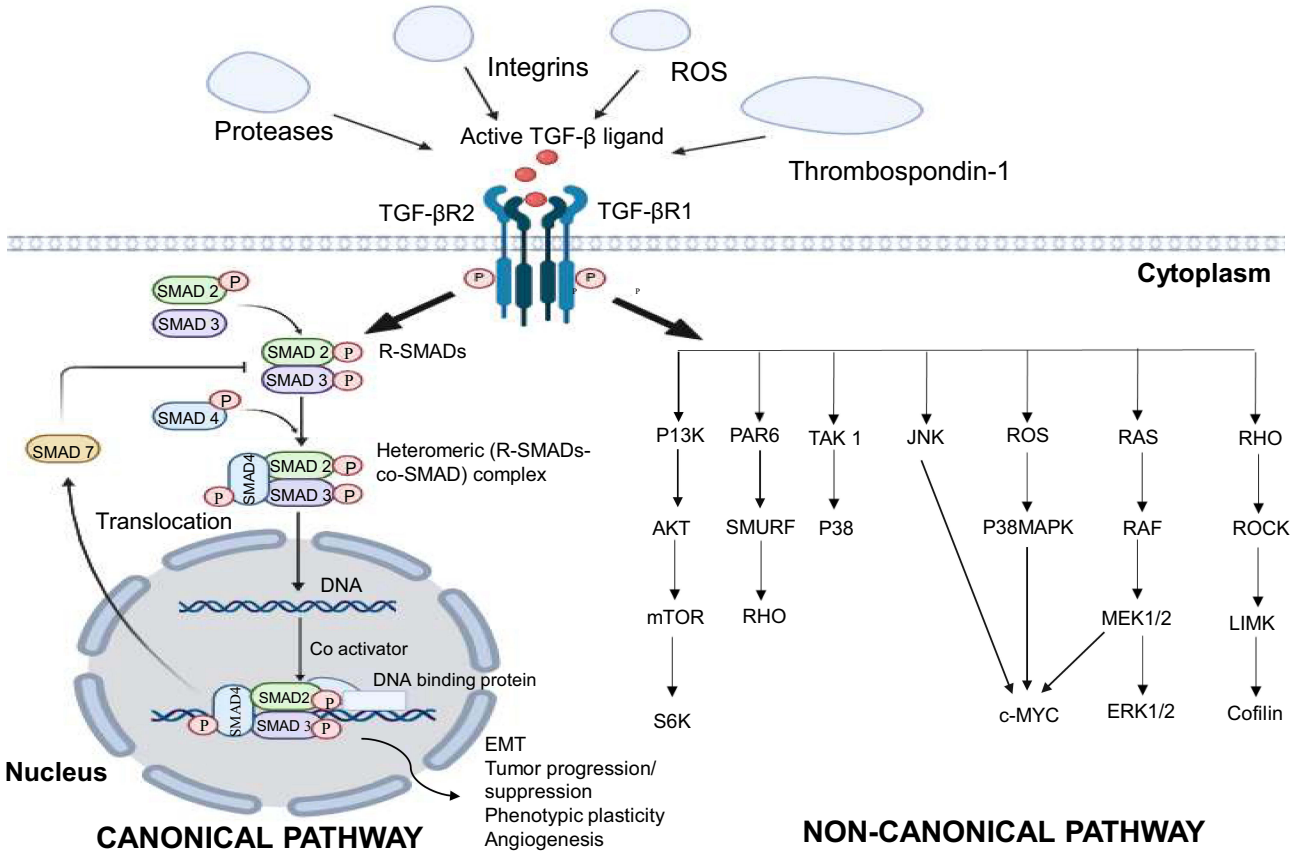

Figure 1 This figure illustrates Canonical (SMAD dependent) and Non-canonical (SMAD independent) TGF-β signaling pathways.[11,75]
Abbreviations: P13K, Phosphoinositide 3-kinase; mTOR, Mammalian target of rapamycin; S6K, ribosomal protein S6 kinase; PaR6, Partitioning-defective protein 6; TAK1, Transforming 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 activing 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 translocation[84] 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

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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]
IFNγ, 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-1α	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; IFNγ, 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, SNAI1/SNAI2 Transcription Factors; ZEB1/ZEB2, Zinc Finger E-Box Binding Homeobox ½; 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 Metallopeptidase 2; MMP-9, Matrix Metallopeptidase 9; IL11, Interleukin 11; MUC1, Mucin 1; PDGF-β, Platelet-Derived Growth Factor Subunit B; CTGF, Connective Tissue Growth Factor; HIF-1α, 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 TβRI.[116] Adaptor protein, ie, SMAD anchor for receptor activation (SARA), microtubules and embryonic liver fodrin (ELF) also mediate SMAD's interaction with TβRI 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 pathway[124] 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 multi-faceted 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]

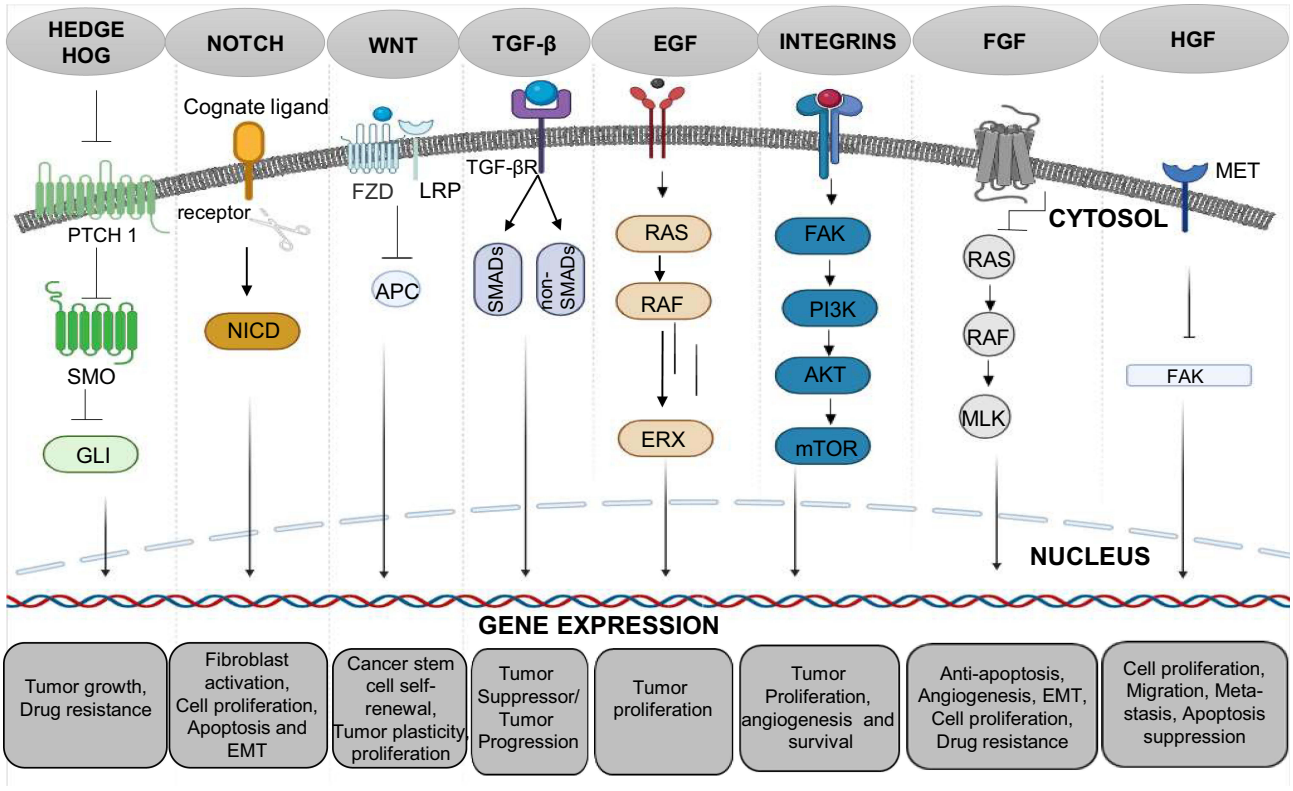

Figure 2 This figure represents the TGF-β signaling pathway cross talk with other related signaling pathways.
Abbreviations: FAK, Focal Adhesion Kinase; NICD, Notch intracellular domain; SMO, Smoothened; FGFs, Fibroblast growth factors; HGF, Hepatocyte growth factor; EGF, Epidermal growth factor; FZDs, 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-3β 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-Kb/Rel Signaling Pathway

NF-κB/Rel signaling pathway is a crucial regulatory network in cellular processes, ie, cell adhesion, senescence, proliferation and survival. NF-κB/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-κB family is classified into two subfamilies; (i) "NF-κB" proteins (p50/NF-κB1 and p52/NF-κB2), (ii) "Rel" proteins (RelA/p65, c-Rel, and RelB).[133] Dysregulation in NF-κB pathway has been linked with various diseases, including arthritis, cancer, cardiovascular diseases, chronic inflammation, asthma, and neurodegenerative disorders.[134]

In the non-canonical NF-κB pathway, the activation is mediated by a specific group of receptors, such as lymphotoxin-α/β or CD40L receptors. These receptors activate NF-κB-inducing kinase (NIK), which subsequently phosphorylates IKKα. Phosphorylated IKKα then phosphorylates the carboxy-terminal residues of NF-κB2 p100, leading to the activation of RelB. NF-κB2 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-κB 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-κB 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-κB. This interaction results in the nuclear translocation of NF-κB dimers, where they can influence gene expression related to inflammation, cell survival, and proliferation.[135]

In cancer, TGF-β/NF-κB 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-κB 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-κB 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-κB 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-κB 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-κB and β-catenin and

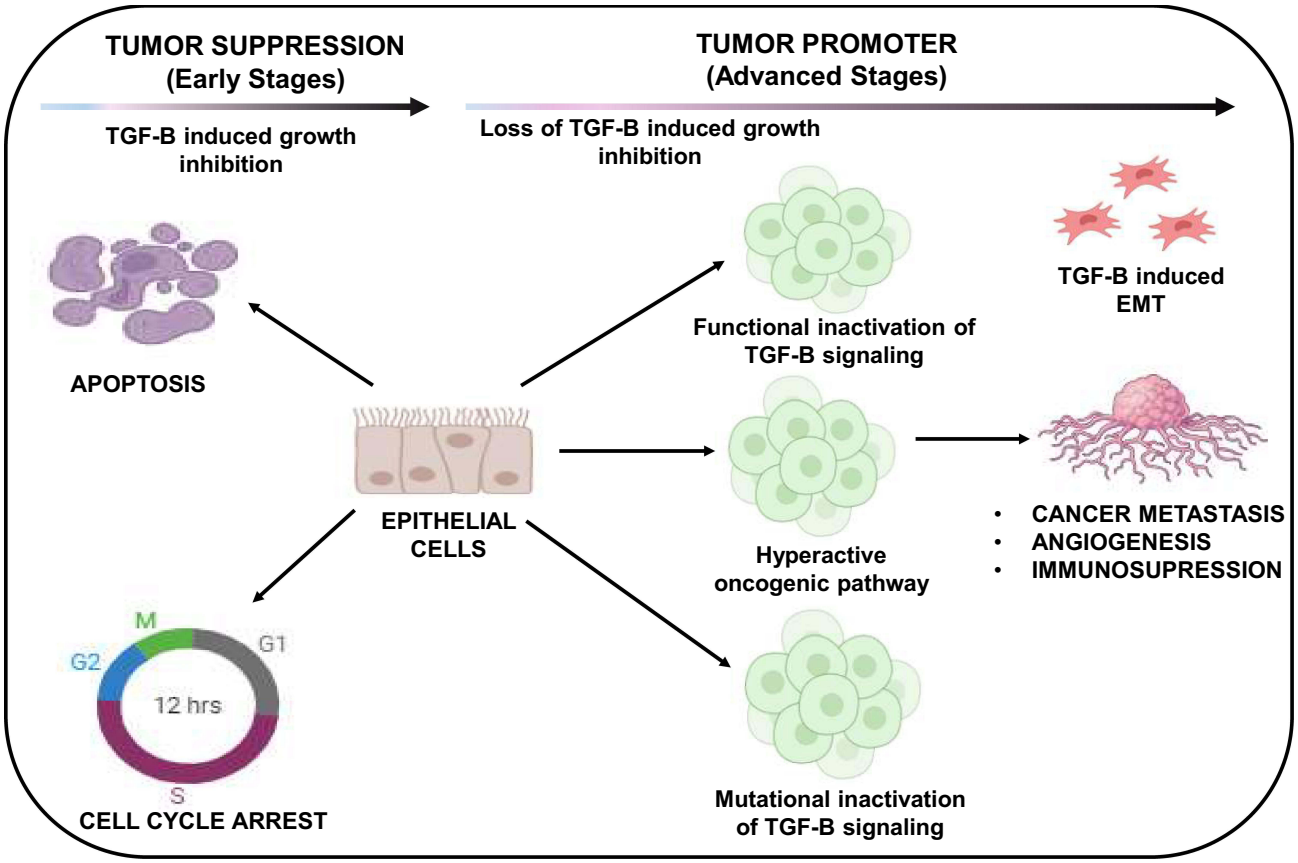

Figure 3 Dual role (early stage; suppression and later stage; promotion) of TGF-β in pancreatic cancer.
Abbreviations: EMT, Epithelial–mesenchymal transition; TGF-B, Transforming Growth Factor Beta.

JNK pathways. This in return 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

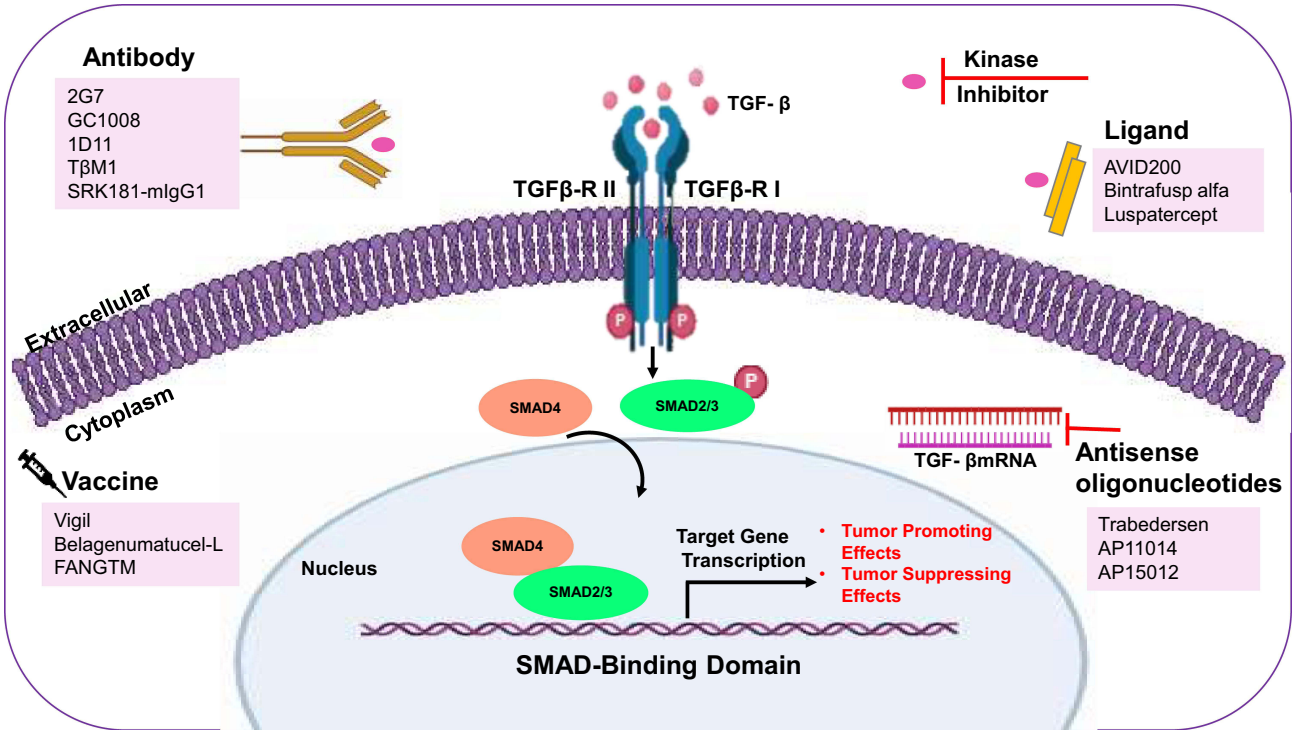

Figure 4 Diagrammatic summary of therapeutic strategies and agents (light purple) targeting the TGF-β signaling pathway. Target gene transcription only indicates the effects regulated by the TGF-β pathway.
Abbreviations: SMAD-R, Receptor-regulated SMADs; P, Phosphorus; SMAD-2, Mothers against decapentaplegic 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-βII.[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-β2, 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 TβRI 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 TβRI 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 TβRI 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 ASOa 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-βII 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-βI 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-β2, 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, TβM1, a monoclonal antibody targeting TGF-β1, 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 TβRI 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 inhibitors[210] (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-TGFβ Therapeutic and Combined Strategies in Pre-Clinical Development

Class	Drugs	Target	Type of Cancer	References
Ligand Trap	sBetaglycan P17, P144 Cisplatin +/− Bintrafusp alfa Bintrafusp alfa + and PD-L1 Brachyury-TRICOM Ado-trastuzumab Emtansine	Pan-TGF-β Pan-TGF-β TGF-β RII and PD-L1 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	TβRII/ALK5 ALK5 TβRI (ALK5) TGF-β TGF-β RI Tubulin TGF-β RI PD-L1 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	AP11014 AP12009 (Trabedersen) GC1008 (Fresolimumab) LY2382770 P144	TGF-β1 TGF-β2 mRNA TGF-β1, β2, β3 TβRI/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 (Gemogenovatucel-T) Vigil + nivolumab Vigil + chemotherapy	TGF-β2 TGF-β1, β2	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*)

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Table 2 (Continued).

Class	Drugs	Target	Type of Cancer	References
Monoclonal Antibody	CAT-192	TGF-β	Sclerosis,	[211]
	LY238770	TGF-β	Hyperkeratosis, Myelofibrosis	
	GC1008	TGF-β	Trabeculectomy, renal fibrosis Diabetes, Idiopathic	
	Fresolimumab+SBRT	TGF-β1, β2, β3	Pulmonary Fibrosis, Glomerulosclerosis (FSGS)	
		RT	Stage Ia/Ib NSCLC	

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 TGFβI and TGFβII 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-BI pathway which leads to stimulation of ITGB1/FAK/Src/AKT. In TNBC patients, the TGF-B inhibitors are potent agents to alleviate the pro-metastatic changes stimulated by TGF-B signalling.[236]

Other TGF-B 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-B1. The second study shows that the inhibitor was able to block MMP-9 expression induced by either EGF or TGF-B 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-B Antagonists

To use anti-TGF-B therapies in controlling the development of various types of cancer, the dual role of TGF-B needs to be understood better. However, the ubiquitous nature and function of TGF-B (which regulates various physiological processes in cells) limits its mechanistic understanding. Therefore, the limited grasp of the complex dual nature of TFG-B (as a tumor suppressor and tumor promoter) is a challenge in the development of TGF-B antagonists for cancer therapy. Numerous combination therapies and TGF-B pathway inhibitors are being explored, yet the varied effects of TGF-B 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-B 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-B, 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-B 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-B, 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-B enhances immunotherapy and overcomes resistance by targeting other pathways like (PI3K/AKT or MAPK) along with the TGF-B pathway, reducing metastasis, and also offering tailored treatment based on tumor development and enhancing drug delivery via nanoformulations. In conclusion, TGF-B 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-B 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.

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Disclosure

The authors report no conflicts of interest in this work.

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