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Review Article

A Mini Review on the Multifaceted Role of TGF-β in Metastasis Progression: Molecular Mechanisms and Novel Therapeutic Strategies

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ABSTRACT

Transforming growth factor-beta (TGF-β) plays a dual role in tumor regulation and metastasis. In the early stages of cancer, TGF-β functions as a tumor suppressor, maintaining cellular homeostasis. However, as cancer progresses, this cytokine can promote tumor invasion and metastasis by modulating the tumor microenvironment and immune responses. TGF-β regulates multiple cellular functions, including growth control, differentiation, apoptosis, and signaling, through SMAD and non-SMAD pathways. In cancer, TGF-β signaling activates the epithelial-mesenchymal transition (EMT), leading to decreased cell adhesion, increased motility, and enhanced tumor invasiveness. These alterations are also linked to chemotherapy resistance. Additionally, TGF-β stimulates angiogenesis by inducing pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), both of which are essential for metastatic tumor expansion. Furthermore, TGF-β contributes to tumor immune evasion by suppressing T-cell and natural killer (NK) cell activity while promoting regulatory T cells (Tregs). Targeted therapies against TGF-β—including ligand receptor inhibitors, monoclonal antibodies, kinase inhibitors, and antisense oligonucleotides (ASO)—have shown promise in preclinical and clinical studies. However, challenges such as tumor resistance and adverse effects resulting from broad TGF-β inhibition remain. Therefore, ongoing research aims to refine TGF-β-based therapies and integrate them with other treatment modalities, including immunotherapy.

Keywords: Transforming Growth Factor beta (TGF-β); Metastasis; Cancer; Antineoplastic Agents

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Introduction

Cancerous transformations are part of a series of complex changes in cells and tissues that occur in response to internal and external stimuli (1). These modifications enhance the tumor's capacity to penetrate and spread, in addition to increasing cell growth and proliferation. Numerous cytokines, including transforming growth factor-beta (TGF-β), play a major role in tumor regulation and metastasis from several angles (2).

TGF-β is one of the key cytokines involved in regulating cellular and tissue activities. This cytokine

participates in various processes, including cell growth regulation, differentiation, apoptosis, and cell-to-cell interactions (3). The TGF-β family includes inhibitors, activins, bone morphogenetic proteins (BMPs), and growth differentiation factors (GDFs) (4). Under normal circumstances, TGF-β functions as a tumor suppressor, promoting cellular equilibrium and homeostasis (5).

Nonetheless, TGF-β has a dual function in diseases such as cancer (6). In the early stages, TGF-β suppresses tumors, but as cancer progresses, it can promote invasion and metastasis by influencing the immune system and the tumor microenvironment (7). Furthermore, TGF-β-related signaling pathways may lead to reduced expression of epithelial markers such as E-cadherin and increased expression of mesenchymal markers such as vimentin and N-cadherin (8). Although epithelial-mesenchymal transition (EMT) is essential for normal embryonic development, in cancer progression, it is hijacked to facilitate tumor invasion and metastasis (9).

There are three isoforms of TGF-β: TGF-β1, TGF-β2, and TGF-β3. Cells produce TGF-β from precursor molecules (10). TGF-β homodimers interact with latency-associated peptides (LAP) to form small latent complexes (SLCs). Upon binding to the extracellular matrix, proteolysis activates these latent complexes, enabling TGF-β to bind to its receptors (TGFbRI, TGFbRII, and TGFbRIII) and initiate intracellular signaling (11). This signaling, primarily mediated by suppressor of mothers against decapentaplegic (SMADs), results in changes such as enhanced metastatic spread, alterations in cellular markers, and differentiation, all contributing to cancer formation and progression (12).

Given the intricate role of TGF-β in metastasis, the primary goal of this review is to provide a concise and informative review of the molecular mechanisms of TGF-β in metastasis and its implications for cancer therapy.

Molecular mechanisms of TGF-β in metastasis

SMAD and non-SMAD signaling pathways

SMADs are intracellular signaling proteins and transcription factors within the TGF-β signaling family (13). Activated TGF-β complexes bind to type 2 serine/threonine kinase receptors. TGFbRII then recruits and phosphorylates TGFbRI (14). Zhang et al. demonstrated that in the absence of TGF-β, type 1 and 2 receptors exist as monomers on the cell surface. SMAD2/3 (R-SMADs) are phosphorylated by TGFbRI and subsequently form a heteromeric complex with SMAD4 (coSMAD). This complex functions as a transcription factor for numerous genes after translocating to the nucleus (15, 16), including the activation of the mitogen-activated protein kinase 8 (MAPK8) pathway, which triggers apoptosis (14). SMAD7—an inhibitory SMAD (i-SMAD) that can interact with TGFbRI and prevent SMAD2/3 activation—E3 ligases (enzymes that ubiquitinate and degrade SMAD2/3 and SMAD4), and SMAD phosphatases (which dephosphorylate SMAD2/3 and SMAD4, leading to their inactivation) are some of the mechanisms that regulate this signaling pathway (11, 17).

Beyond the SMAD-mediated TGF-β signaling route, TGF-β receptors can also activate other intracellular signaling pathways. A significant portion of the complexity and diversity of cellular responses to TGF-β is attributed to these non-SMAD pathways (18). The PI3K-AKT-mTOR pathway is essential for controlling cell division, survival, and metabolism. By interacting with PI3K's p85 subunit, TGF-β can activate the protein, which in turn triggers AKT and subsequently mTOR. mTOR then regulates protein translation (19).

The RhoA/ROCK pathway is crucial for migration, cell motility, and cytoskeletal remodeling. TGF-β can activate ROCK via the RhoA GTPase, leading to cytoskeletal modifications and enhanced cell motility (20). Additionally, three key MAPK pathways—p38, JNK, and Erk—can be activated through phosphorylation and activation of MAPK kinases (MKKs), which are regulated by activated TAK1. The MAPK pathways play a critical role in TGF-β-induced cellular processes: Erk activation enhances gene expression and cell proliferation, while JNK and p38 MAPK activation can lead to cell death (21).

Furthermore, TGF-β can activate other signaling pathways, including NF-κB, Wnt, and Notch, which regulate gene expression, cell division, and cell death in various ways (18).

Epithelial-mesenchymal transition (EMT)

In healthy cells, EMT is essential for tissue repair and development. On the other hand, TGF-β stimulates EMT in cancer cells at advanced stages of the disease, aiding the growth, metastasis, and advancement of tumor cells (22). Vimentin, E-cadherin, and N-cadherin are among the markers that alter throughout the EMT process, causing a decrease in cell adhesion as well as a loss of cell polarity (23). Furthermore, as mesenchymal traits are acquired, there is an increase in motility, invasiveness, and, eventually, metastasis (22, 23).

Research has indicated that chemotherapy resistance is linked to mesenchymal cancer cells (24). Current research casts doubt on EMT's ability to trigger metastasis. EMT is not necessary for the spread of lung cancer or pancreatic adenocarcinoma, according to studies conducted by Zheng et al. and Fischer et al. These findings suggest that EMT may only be a factor in chemoresistance (25, 26).

TGF-β-induced EMT is mediated by a complex set of signaling pathways and regulatory molecules. TGF-β stimulates the production of SNAIL, ZEB, and TWIST—proteins known to suppress epithelial markers and enhance the expression of mesenchymal genes—via

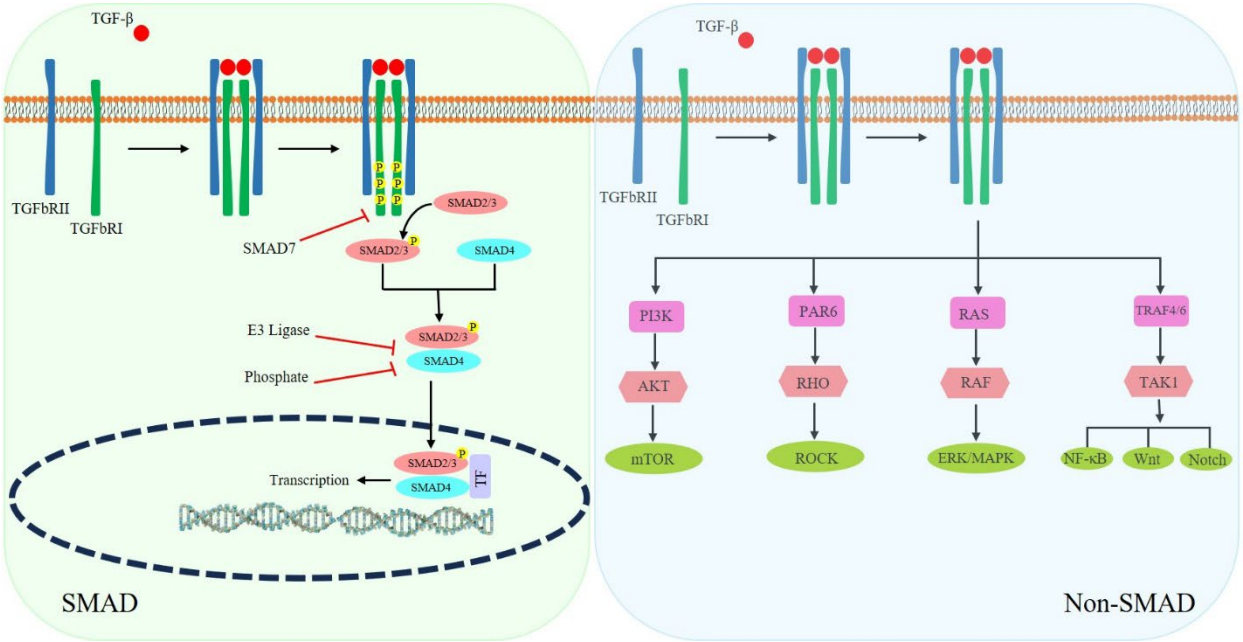

Figure 1. TGF-β functions through two main signaling pathways: **SMAD pathway:** In this pathway, the TGF-β molecule first binds to its cell surface receptors, namely TβR II, and activates this receptor. Then, TβR II recruits TβR I, and together they phosphorylate receptor-associated SMAD proteins (R-SMAD). This phosphorylation leads to the formation of an R-SMAD complex with co-SMAD proteins. Subsequently, this complex is transported into the cell nucleus, where it regulates the transcription of specific genes, such as the CAGA gene. This pathway directly plays a role in gene regulation and cellular changes. **Non-SMAD pathway:** This pathway is activated when TGF-β binds to other downstream factors. These factors include proteins such as SHC/GRB2/SOS, TRAF4/6, PAR6, and PI3K. Unlike the SMAD pathway, which directly influences gene regulation, the non-SMAD pathway operates through more complex mechanisms involving a variety of proteins and can regulate processes such as cell survival, cell migration, and immune responses.

the SMAD signaling pathway (27). Additionally, non-SMAD signaling pathways, including PI3K/Akt and MAPK pathways (such as ERK, JNK, and p38), are activated by TGF-β. By regulating the expression and activity of transcription factors linked to EMT, as well as downstream effectors, these pathways enhance the EMT process and converge with SMAD pathways (18).

TGF-β also regulates mesenchymal and epithelial markers. Mesenchymal markers, including vimentin, fibronectin, and N-cadherin, are increased, whereas epithelial markers, such as E-cadherin and cytokeratins, are downregulated (28). Notably, TGF-β-induced EMT can be influenced by extracellular matrix components, cytokines, and growth factors (29). The crosstalk between cancer and stromal cells in the tumor microenvironment can further promote the EMT process and contribute to tumor progression and metastasis (28, 29).

The Role of TGF-β in Promoting Metastasis

The intricate and multi-step process of metastasis includes the following steps: a) invasion; b) intravasation, survival, and extravasation from the bloodstream; and c) establishment of new colonies in distant organs (30). Changes in the EMT process of cancer cells and alterations in the tumor microenvironment are two key variables that enable cancer stem cells (CSCs) to leave the original tumor. As previously discussed, EMT is a complex process. TNF-α, IL-6, and chemokines 4/12 are examples of factors that can further promote EMT within the tumor microenvironment (31). Moreover, TGF-β and TNF-α enhance EMT (30, 31).

Additionally, growth hormones such as FGF and IGF are secreted by cancer cells, driving the tumor microenvironment toward high interstitial fluid pressure (IFP), hypoxia, and acidity (32). These conditions facilitate the remodeling of the extracellular matrix (ECM) surrounding the tumor, allowing cancer-associated fibroblasts (CAFs) to promote metastasis more efficiently (30, 32). TGF-β plays a crucial role in metastasis to various organs, including the lungs, prostate, liver, and bones (33).

The delicate balance of bone remodeling is disrupted by cancer cells that have metastasized to the bone, fostering the formation of bone tumors (34). TGF-β secreted from bone by osteoclasts enhances PTHrP signaling in osteoblasts, leading to reduced OPG expression and increased RANKL expression (34, 35). This imbalance favors RANKL, which increases osteoclastic activity, thereby triggering further production of TGF-β. This creates a positive feedback loop that drives bone metastasis (36).

The Multifaceted Role of TGF-β in Angiogenesis

Angiogenesis is the process by which pre-existing blood vessels divide to generate new ones. It plays a crucial role in both physiological and pathological conditions, such as wound healing and tumor growth (37). TGF-β stimulates angiogenesis through multiple mechanisms. It induces the expression of numerous pro-angiogenic factors, including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) (38). Additionally, it directly influences endothelial cells, promoting migration and proliferation—two essential phases of angiogenesis (39).

TGF-β regulates the production and accumulation of extracellular matrix (ECM) components, such as glycoproteins, collagen, and fibronectin (40). This complex ECM network provides structural support to newly formed blood vessels (41). Furthermore, TGF-β facilitates the recruitment of smooth muscle cells (SMCs) and pericytes to developing arteries. These supporting cells are essential for maintaining vascular stability and enhancing the maturity and functionality of blood vessels (42).

Additionally, TGF-β modulates the expression of cell adhesion molecules and junctional proteins in endothelial cells, regulating their interactions with neighboring cells. This regulation of cell-cell interactions is critical for coordinating endothelial cell behavior during angiogenesis (43). Overall, TGF-β plays a multifaceted role in angiogenesis by orchestrating various cellular processes involved in blood vessel formation and remodeling.

TGF-β Promotes Immune Evasion

Under normal circumstances, T lymphocytes and natural killer (NK) cells can identify and destroy cancer cells. However, TGF-β helps tumor cells evade the body's immune system. Although the exact mechanism of TGF-β remains unknown, several potential pathways have been proposed, which we will briefly discuss. Gorelik et al. demonstrated that the primary role of TGF-β is to negatively regulate T lymphocytes. TGF-β can impede T and B cell growth, reduce immune factor synthesis by B lymphocytes, affect NK cell function, and hinder Th1 cell development (44).

Regulatory T cells (Tregs) play a crucial role in tumor immune evasion. TGF-β stimulates FoxP3 expression and induces Tregs that suppress immune responses against tumors (45). Dendritic cells (DCs) prime and activate T cells during the immune response. TGF-β promotes apoptosis and prevents DC migration, facilitating immune evasion (46). Tumor-associated macrophages (TAMs) are polarized by TGF-β from an M1 anti-tumor phenotype to an M2 pro-tumor phenotype (47). Additionally, TGF-β can inhibit neutrophil degranulation and promote the synthesis of interleukin 10 (IL-10).

Overall, TGF-β plays a significant role in tumor immune evasion by regulating multiple immune cells and promoting immunosuppressive factors (48).

Targeted TGF-β Therapy Strategies, Challenges, and Future Prospects

Therapeutic Strategies

TGF-β is a molecule with dual functions in cancer development. It may act as a tumor growth inhibitor or, in some cases, facilitate disease progression. Thus, developing effective cancer treatments requires a thorough understanding of the TGF-β signaling system. Fortunately, scientific research in this field has advanced significantly. Several investigations have demonstrated that pharmacological therapies targeting different components of the TGF-β signaling system have produced encouraging outcomes in preclinical and clinical trials. TGF-β-based treatment strategies can generally be categorized into five groups: 1) Ligand traps; 2) Monoclonal antibodies; 3) Receptor kinase inhibitors; 4) Antisense oligonucleotides; and 5) Aptamers (peptide- and nucleotide-based) (49).

A comprehensive assessment of several factors must be conducted to optimize therapeutic applications. Key considerations include evaluating TGF-β levels and isoforms, the expression of genes responsive to TGF-β, the types of cells influenced by TGF-β, genetic analysis of tumors for expression and mutations in TβRII, TβRI, and SMADs, and the extent of host cellular responses (49, 50).

The monoclonal antibody 1D11, which binds to all three isoforms of TGF-β and inhibits its activity to prevent bone tumor development, was developed for use in mice (51). For potential patient applications, Genzyme Co. has developed human versions of these neutralizing antibodies, including Lerdelimumab, Fresolimumab, and Metelimumab (52). Numerous TβRI kinase inhibitors have been designed and are currently in clinical trials for cancer treatment. The TβRI kinase inhibitor Galunisertib (LY2157299) has demonstrated satisfactory safety and tolerability in early-phase trials involving Japanese patients with advanced solid malignancies (53). Furthermore, it is currently being investigated in combination with immunotherapy and anti-PD-1 antibodies such as nivolumab and durvalumab (Clinical Trials: NCT02423343) (54). Studies have shown that TβRI inhibitors, such as SM16, and TGF-β neutralizing antibodies, such as 1D11, synergistically prevent metastasis when combined with OX40 agonist antibodies (55). In melanoma and glioma models, the TβRI inhibitor SD208 has demonstrated efficacy in halting bone metastases and slowing the progression of osteolytic lesions by blocking the TGF-β/SMAD pathway (56).

Antisense oligonucleotide (ASO) therapies represent another cutting-edge treatment strategy that can inhibit TGF-β-induced metastasis by reducing its production. ASOs bind to corresponding RNA sequences, leading to mRNA degradation and decreased TGF-β protein synthesis (57). For instance, Trabedersen (AP 12009) is an ASO specific to human TGF-β2 coding RNA that inhibits the production of this isoform, thereby preventing TGF-β-driven metastasis (58). Another ASO, Belagenpumatucel-L (Lucanix), has demonstrated anti-tumor efficacy in non-small cell lung cancer (NSCLC) by inhibiting TGF-β2 to reduce its immunosuppressive effects (59).

Due to their specificity in target binding, small molecule peptides offer another therapeutic approach that can suppress the TGF-β signaling pathway while minimizing adverse effects compared to other treatment modalities (60). Han Kang K et al. demonstrated that the TAT-SNX9 peptide exhibits anti-fibrotic properties against TGF-β in mouse and human lung fibroblast cells (61). SNX9 inhibits the TGF-β signaling pathway by binding to SMAD3 and preventing its nuclear translocation (57). Additionally, Trx-SARA inhibits the formation of the SMAD2-3/SMAD4 complex. Studies have also shown that Trx-SARA can suppress the TGF-β-induced epithelial-mesenchymal transition (EMT) process (62).

Advantages and Disadvantages

Each of these treatment approaches has unique benefits and drawbacks. While receptor and ligand inhibitors broadly block TGF-β signaling throughout the body, they also pose a risk of adverse effects. Monoclonal antibodies, on the other hand, offer a more precise means of targeting TGF-β in specific cells or tissues, potentially reducing side effects. However, the high cost and complexity of monoclonal antibody production present additional challenges.

Challenges

The development of effective TGF-β therapies faces several hurdles. Key challenges include:

· **Improving the specificity of TGF-β inhibitors** – Current inhibitors block all three isoforms of TGF-β, potentially leading to side effects. Developing isoform-specific inhibitors is essential.

· **Overcoming resistance to TGF-β therapy** – Some tumors exhibit resistance to TGF-β-targeted treatments. Understanding these resistance mechanisms and formulating strategies to counteract them is crucial.

· **Developing combination therapies** – TGF-β therapy may be more effective when paired with other treatments such as immunotherapy or chemotherapy. Identifying optimal therapeutic combinations is a priority.

Future Prospects

Researchers continue to explore novel approaches to improve TGF-β therapies. Some of the most promising areas of investigation include:

· **Nano-based therapies** – Nanotechnology can enhance the targeted delivery of TGF-β inhibitors to tumors or specific cells, thereby minimizing side effects and improving efficacy.

· **Gene therapy** – Gene therapy offers a potential long-term solution by introducing genes into cells that express TGF-β inhibitors.

· **Personalized medicine** – Scientists are working to identify patients who are most likely to benefit from TGF-β therapy, ensuring that treatments are tailored to individual needs.

TGF-β remains a promising target for cancer treatment. However, further research is necessary to develop more effective therapies. Some of these treatments are currently undergoing clinical trials, with the hope that they will receive approval for broader use in the coming years.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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