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Review Article

Mechanism of TGFβ in Bone Metastases and its Potential Therapeutic Uses

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Abstract

Complications associated with advanced cancer pose a clinical challenge, particularly when bone metastases are involved, as they can worsen the prognosis and reduce the patient's chances of survival. Solid tumors, such as those originating from the breast, prostate, and lungs, can potentially metastasize to bone. Mineralized bone matrices contain potent growth factors and cytokines. The bone microenvironment is distinctive, furnishing prolific soil for cancer cell proliferation. Following tumor-induced bone destruction by osteoclast, the Transforming Growth Factor (TGFβ) is released from the mineralized bone matrix. It is one of the most abundant growth factors released from the bone matrix. TGFβ stimulates tumor cell secretion of factors that accelerate bone resorption and stimulate tumor cell colonization.

Consequently, TGFβ is essential for fueling cancer's vicious cycle of cancer growth and bone destruction. In addition, TGFβ promotes Epithelial-Mesenchymal Transition (EMT), increasing cell invasiveness, angiogenesis, and metastasis progression. Emerging evidence demonstrates that TGFβ inhibits immune responses, allowing opportunistic cancer cells to evade immune checkpoints and promote bone metastasis. By inhibiting TGFβ signaling pathways, cancer progression in the bone could be broken, EMT could be reversed, and immune response could be improved. However, the dual function of TGFβ as both a tumor suppressor and an enhancer pose a formidable obstacle to developing therapeutics that target TGFβ signaling. This review delves into the significance of TGFβ in the advancement of cancer and bone metastases, in addition to examining the current therapeutic prospects of TGFβ pathway targeting.

Keywords: Bone metastases programmed cell death ligand (PD-L1); Bone resorption; Immune cells; Immune checkpoint inhibitors; Transforming growth factor-β (TGFβ); TGFβ Blockade

Introduction

Cancer is the primary cause of death in industrialized nations. Certain solid malignancies, including breast, prostate, and lung cancer, tend to metastasize to the bone. Each year, more than 1.5 million patients with cancer develop bone metastases worldwide. Breast cancer is the primary cancer diagnosed in women, with around 1.38 million new cases accounting for 23% of all cancer cases. Breast cancer is also the leading cause of cancer-related deaths in women worldwide [1,2]. Invasive breast cancer history is prevalent among almost 3 million women in the US, with over 226,870 new cases diagnosed in 2012 [3]. It is estimated that 70% of patients with advanced breast cancer develop bone metastases [4-6]. Prostate cancer is the second most frequent cancer diagnosis in men and the fifth leading cause of death worldwide [7]. Bone metastases are prevalent in about 85% of advanced prostate cancer patients and 40% of lung and kidney cancer patients. The majority of malignancies predominantly metastasize to the axial skeleton [8], especially the spine (87%), pelvis (63%), cranium (35%), and

ribcage (77%), as well as the proximal region of the humeri and femora (53%), as opposed to the distal regions of the appendicular skeleton (1%) [9].

Patients with bone metastases are susceptible to skeletal complications known as skeletal-related events (SREs). These events include pathological fractures, pain, spinal cord compression, hypercalcemia, complications from bone surgery, and radiation therapy. SREs are linked to impaired mobility, diminished quality of life, increased mortality, and increased healthcare costs [10]. Standard Antiresorptive treatments are the standard of care for patients with bone metastases and SRE. These drugs can reduce skeletal morbidity and delay SRE but do not eradicate the disease [6,11]. With the improvement in cancer therapy, patients with cancer who develop bone metastases can live long after their diagnosis, despite experiencing significant morbidity. To achieve the ultimate objective of preventing or curing bone metastases, it is essential to develop more effective therapies. The bone microenvironment is distinct and provides ideal conditions for cancer growth. The mineralized bone matrix releases numerous growth factors and cytokines during osteoclastic bone resorption. Transforming growth factor β (TGFβ) is the most prevalent of these factors in the bone matrix. In addition to activins, inhibins, and Bone Morphogenetic Proteins (BMPs), the TGFβ superfamily includes other factors implicated in bone homeostasis, such as Bone Morphogenetic Proteins (BMPs). Bone-derived TGFβ is activated by proteolytic cleavage, pH variations in the microenvironment, or by interacting with integrins. In addition, TGFβ stimulates the production of pre-osteolytic and osteolytic factors by tumors, which promotes additional bone resorption [12-14]. This reveals TGFβ as a significant factor that drives the feed-forward vicious cycle of bone tumor proliferation. Therefore, inhibiting the release, production, and/or signaling of TGFβ is a propitious treatment for bone metastasis. Several inhibitors of TGFβ have been developed in recent years, including TGFβ receptor kinase inhibitors, TGFβ neutralizing antibodies, soluble receptor decoys (Fc fusions), and TGFβ antisense oligonucleotides [15]. Several of these are currently in clinical trials for a variety of disease indications, with a focus on their potential as cancer therapies, including those for bone metastases. This review will focus on the function of transforming growth factor in bone metastasis and the therapeutic use of new TGFβ inhibiting drugs and biologics for the treatment of bone metastases.

TGFB Receptors, Ligand and Signaling

The transforming growth factors (TGFβs) are broadly expressed in embryonic and adult normal tissues. Its original name came from its capacity to cause cancerous behavior in healthy fibroblasts. The transforming growth factor β family encompasses a group of proteins that are secreted in homodimeric and heterodimeric forms and are responsible for regulating the differentiation of the vast majority of cell lineages, along with multiple aspects of cell and tissue physiology in multicellular eukaryotes. In mammalian cells, 33 genes are responsible for encoding TGFβ. It is a multifunctional cytokine that has a role in several biological functions, including cell growth, including cell growth and differentiation, inflammation, Epithelial-Mesenchymal Transition (EMT), angiogenesis, immune responses, apoptosis, and autophagy [16-18]. Chronic fibrosis, cancer, and cardiovascular diseases, and several other disorders have been associated with the dysregulation of TGFβ functions [19,20].

The TGFβ superfamily consists of about 33 subfamilies of protein ligands based on sequence similarity and function. TGFβs, Bone Morphogenetic Proteins (BMPs), activins, inhibins, NODAL, Anti-Müllerian Hormone (AMH), and growth and Differentiation Factors (GDFs), are members of the TGFβ superfamily. All the ligands are synthesized in the form of precursors, containing large N-terminal prodomain that plays a crucial role in ensuring the proper folding and dimerization of proteins. Upon cleavage, the mature ligands come into being, forming either homodimers or heterodimers, which remain bound by disulfide bonds. In some instances, the pro-domain remains non-covalently associated with the mature protein after secretion. The secretion of Transforming Growth Factor β (TGFβ) occurs in the form of a precursor that remains in an inactive state. Following its secretion, TGFβ undergoes binding with its pro-domain, which is also known as Latency Associated Protein (LAP), resulting in the inactivation of this ligand. This inactivation of TGFβ facilitates its association with inhibitory latent TGFβ binding proteins (LTβPs). These LTβPs are responsible for targeting the complex to the Extracellular Matrix (ECM), where latent TGFβ is sequestered. Three isoforms of TGFβ have been identified in humans: TGFβ1, TGFβ2, and TGFβ3. All three of these isoforms have similar signaling properties, The signaling mechanism of these three isoforms is comparable, but their tissue-specific expression levels vary [21,22]. All the ligands use essentially the same signaling mechanism. Each ligand has a corresponding type I and type II serine/threonine kinase receptor. Both the receptors are transmembrane kinases with comparable similarities in structure; including a glycosylated, disulfide-rich ectodomain of about 100 amino acids, a short juxtamembrane sequence, a transmembrane region, and a cytoplasmic kinase with its 11 subdomains organized in an N- and a C-lobe. In response to ligand interaction, the type II receptor kinase phosphorylates a short Gly-Ser-rich motif, the GS domain, in the juxtamembrane sequences of type I receptors [23]. It has been shown that the type I-type II receptor complex requires extra co-receptors for optimum ligand binding in certain cases. Phosphorylation of C-terminal SMAD residues are a signaling mechanism used by TGFβ and other members of this family. In the activated receptor complex, the type I receptor is phosphorylated on many serines and threonines in a highly conserved glycine- and

serine-rich domain near the membrane-spanning region. When the type I receptor kinase is phosphorylated, it becomes active and generates a binding site for its downstream substrates, the receptor regulated SMADs (RSMADs). The signaling process which is mediated by TGFβ ligands is transduced by complexes of type I and type II transmembrane serine-threonine kinases on the cell surface. A bi-dimeric receptor complex is formed when TGFβ attaches to its receptors on the cell surface, which includes the TGFβ type I receptor (TβRI, also known as ALK-5) and the TGFβ type II receptor (TβRII). Upon binding of the ligand to the type II receptor, it phosphorylates serine and threonine residues in the type I receptor, which ultimately propagates the signal through Smad activation [24].

Smad Mediated Signaling

The effects of the TGFβ family proteins are conveyed by a group of receptor-activated mothers against decapentaplegic homolog (SMAD) transcription factors. These transcription factors modify, regulate, and establish connections between many signaling inputs, notably TβRI phosphorylation, and target gene regulation and expression. The Smad family members can be classified into three main groups. 1) the receptor-activated Smads (R-Smads) These are Smads 1,2,3,5 and 8. 2) co-mediator Smads (Co-Smad), this is Smad4, 3) inhibitor Smads, which include Smad6 and 7 [25]. ALK1/2/3/6 are responsible for phosphorylating Smad1/5/8 after BMP or GDF activation, ALK4/5/7 are responsible for phosphorylating Smad2/3 after TGFβ, NODAL, or Activin signaling [24]. The binding of active TGFβ to the TGFβ receptor type II (TRII) results in the recruitment and activation of TGFβ receptor type II (ALK5). When ALK5 phosphorylates R-Smad2/3, they join with the common mediator Smad (also called Smad4 or co-Smad) to form a heterodimeric complex and translocate to the nucleus[26,27]. Later, in the nucleus, the Smad complexes will perform the function of a transcriptional regulator at specific DNA locations. This will allow them to influence the expression of the target gene. Therefore, SMAD4 plays a pivotal role in the signaling pathways that follow all the ligands, as it is necessary for many but not all responses.[28,29]. The Smad complex collaborates with a number of other transcription factors [30,31] so that it can attain a high binding affinity for the Smad-Binding Elements (SBE) that are located in the promoters of TGFβ target genes. There are many other families of transcription factors that are partners of Smad [31], including forkhead, homeobox, zinc finger, AP1, Ets, and basic helix-loop-helix. In addition, in order to regulate gene transcription, the Smad complex is responsible for recruiting co-activators, such as p300 and CREB binding protein, as well as co-repressors, such as retinoblastoma-like 1 protein [26,27,31]. Therefore, even though Smad proteins are, by nature, transcriptional activators, the result of the transcription process involving their target genes is frequently contingent on the transcriptional partners that are connected with Smads [32].

A novel branch of the TGFβ signaling pathway has been recently found. This branch is characterized by activating the R-Smads, Smad1/5, by ALK5, which ultimately results in TGFβ -induced anchorage-independent growth and cell migration [33,34]. In addition, TGFβ has the ability to alternatively activate the R-Smads, specifically Smad1/5/8, by way of the TGFRI ALK1, which is primarily expressed in endothelial cells [35]. In fact, signaling via TGF-/ALK1 encourages endothelial cell proliferation and migration, whereas signaling via TGF-/ALK5 discourages these processes [36,37]. A mechanism of negative feedback loop control for TGFβ signaling is carried out by inhibitory Smads, including Smad6 and Smad7. Smad4 and Smad6 contend for Smad1 binding, whereas Smad7 and Smad6 induce Smurf to deactivate signaling at the TGFβ and bone morphogenetic protein (BMP) receptor level [38]. Yan et al., elaborated on a previously undiscovered mechanism by which Smad7 inhibits TGFβ signaling at the Smad level. During the process of TGFβ signaling, Smad7 formed oligomers with R-Smad proteins, which then directly reduced the activity of R-Smad. On a mechanistic level, Smad7 competes with Smad4 to connect with R-Smads.It recruits the E3-ubiquitin ligase NEDD4L to activated R-Smads, which ultimately results in the polyubiquitination of R-Smads and their subsequent destruction by the proteasome. In a manner analogous to the oligomerization that occurs between R-Smads and Smad4, the interaction that occurs between R-Smads and Smad7 is mediated by their mad homology 2 (MH2) domains. These findings provide fresh insight into how Smad7 controls the signaling of TGFβ in the cell [39].

Smad-Independent Signaling

Various alternative pathways of TGFβ signaling diverge from the canonical signaling pathway at the receptor level. Apart from the Smad-mediated signaling pathways that are triggered by ligand binding to TGFβ receptors, TGFβ can also activate Smad-independent signaling pathways by interacting and associating with other alternative mediator proteins [40]. The activated TGFβR2/ALK5 receptor complex can activate TRAF6-TAB1-TAK1 and downstream p38 and JNK signaling. Moreover, the receptor complex can also activate PI3K/AKT signaling and contribute to cascades such as Ras/MEK/Erk, Rho/Rock, CDC42/Rac-Pac, and Jak/Stat [41,42]. Additionally, the receptor complex can also activate PI3K/AKT signaling and contribute to Ras/MEK/Erk, Rho/Rock, CDC42/Rac-Pac, and Jak/Stat signaling cascades.

Mitogen-Activated Protein (MAP) kinases, for example, the extracellular signal-regulated kinases (Erk1 and 2), Rho-like GTPase, and p38 and c-Jun amino-terminal kinase (JNK) MAP kinases, are among the different Smad-independent pathways activated by TGFβ. Shc, an adaptor protein, must be recruited

and phosphorylated for Erk MAP kinase activation Shc connects with Grb2, another adaptor protein, and the GTP exchange factor SOS [43]. This protein complex activates Ras to its GTP-bound state as well as the kinase cascade is composed of c-Raf, MEK1 or MEK2, and Erk1 or Erk2. TGFβ also activates the p38 and JNK MAP kinase pathways via the Tumor Necrosis Factor (TNF) receptor-associated factor 6 (TRAF6) and TAK1. TRAF6 binds to the TGFβ receptor complex, then undergoes auto-ubiquitylation, and becomes active. TAK1, also known as MAP3K7 I, is a TGFβ stimulated serine/threonine kinase and a key signaling molecule in the TGFβ-mediated expression of fibronectin and Type I collagen. It is polyubiquitylated and phosphorylated when active TRAF6 is associated with it [41,42]. Active TAK, in turn, activates p38 MAP kinase and JNK. At epithelial cell junctions, TGFβ receptor complexes interact with the polarity protein Par6 and the tight junction protein occludin. At these junctions, the receptor complex phosphorylates Par6, and it is associated with Smurf1. The Par6-Smurf1 complex leads to the ubiquitylation of RhoA, which ultimately results in the dissociation of tight junctions. For the localization of TβRI to tight junctions, the interaction of occludin with TβRI is a necessary requirement. This is critical because it is a prerequisite for TGFβ induced dissolution of tight junctions during epithelial-mesenchymal transition [44]. Consequently, the cellular responses to TGFβ signaling are the result of the dynamic combination of canonical and non-canonical signaling cascades.

TGF-B Signaling And Epithelial-Mesenchymal Transition (EMT)

Epithelial Mesenchymal Transition (EMT) is the program by which epithelial cells lose their epithelial properties and acquire the invasive and migratory characteristics of mesenchymal cells. EMT is a critical program in the natural developmental processes, allowing neuro-ectodermal and epithelial cells to migrate to different sites and generate diverse cell types. It is considered part of the pathological process in diseases like certain carcinoma and fibrosis. This phenotype changes from epithelial to mesenchymal is mostly caused by a group of important transcription factors, most often Snail, Twist, and ZEB. These transcription factors are responsible for the epigenetic repression of epithelial markers, the transcriptional activation of matrix metalloproteinases, and the remodeling of the cytoskeleton.

TGFβ channels the expression of the EMT master transcription factors Snail1/2, ZEB1/2, and Twist by acting through Smad3 [45-48]. The Smad3/4 complex binds directly to the regulatory region of the Snail promoter, inducing transcription. The Smad3/4 complex activates transcription by binding specifically to the Snail promoter's regulatory region. Repression of E-cadherin, claudin, and occludin expression is facilitated by Smad3/4 complexes working together and associating with Snail. [49]. The miR-200 family represses ZEB1 and ZEB2 mRNAs as cells progress through EMT. All five members of the miR-200 family are suppressed by TGFβ, leading to elevated ZEB1 and ZEB2 levels that directly repress miR-200 expression through ZEB1 binding to regulatory regions [50,51]. TGFβ also modulates the expression of MMP2 and MMP9, as well as ECM (fibronectin and collagen) components [52]. Furthermore, TGFβ can activate the expression of EMT transcription factors via alternative splicing [53]. The process of Epithelial-Mesenchymal Transition (EMT) is regulated through a cluster of microRNAs that are responsible for determining cytoskeletal reorganization and alterations in epithelial polarity. This process is directly activated by TGFβ through the Smad/RhoA pathway [54]. During EMT, it has been observed that Smad-independent TGFβ signaling pathways, like the PI3K/Akt/mTOR pathway, lead to an increase in protein synthesis, cell motility, and invasion. Conversely, the inhibition of PI3K, Akt, or mTOR has been shown to prevent full EMT in response to TGFβ. TGFβ has also been found to induce EMT through a process that is involving ubiquitylation and sumoylation. [55]. The Smad3/4 complex regulates the expression of HDM2, which increases the ubiquitylation and degradation of p53, thereby promoting the progression of EMT [56]. The expression of the SUMO E3 ligase PIAS1 is downregulated by TGFβ signaling, leading to a decrease in sumoylated SnoN, an antagonist of TGF-mediated EMT. More recently, Xu et al., reported a new smad2/3-Associated long noncoding RNA (SMASR) which interacts with Samd2/3 to inhibit the expression of TβRI and inactivation of the Smad signaling pathway. In lung cancer cells, TGFB downregulates SMAR through Smad2/3 [57].

Physiology of Bone

Bone performs a number of crucial physiological functions within the human body, including mechanical functions such as locomotion, protection to vital organs, rigid support, and an attachment site for skeletal muscles. Bone also performs several metabolic functions, acting as a repository of essential minerals, especially calcium and phosphorus [58,59]. Bone is a dynamic tissue that undergoes constant remodeling, alternating the processes of bone resorption and bone formation required to prevent the accumulation of bone damage and maintain both the mechanical strength of bone and calcium homeostasis [60]. Bone tissue is primarily composed of type I collagen that has undergone mineralization through hydroxyapatite. The weight composition of bone is approximately 60% mineral, 30% organic matrix, and 10% water. The mineral component is primarily composed of hydroxyapatite crystals, which are naturally occurring calcium phosphate. The organic matrix, on the other hand, is 98% type I collagen and 2% collagenous protein. Non-collagenous proteins, which include growth factors, extracellular matrix proteins, proteoglycans, osteocalcin, osteonectin, osteopontin, and cytokines, make up a minority of bone volume but contribute

significantly to its biological function. Despite not being a major contributor to bone volume, non-collagenous components play an important role in bone growth, activation, and differentiation. They include growth factors and cytokines such as transforming growth factor β (TGFβ), Bone Morphogenetic Proteins (BMPs), the Tumor Necrosis Factors (TNFs), insulin-Like Growth Factor (IGF), interferon-,), the interleukins (ILs), and osteoprotegerin (OPG), and are present in minimal amounts in the bone matrix. These components significantly affect bone cell differentiation, activation, and bone growth [61,62].

The Cellular Component of Bone and Mechanism of Bone Remodeling

Several bone cells are associated with bone homeostasis. These include osteoblasts which are responsible for bone formation, osteoclasts which resorb bone, and osteocytes which are embedded within the bone matrix [63]. Mature osteoclasts are multinucleated cells originating from the same hematopoietic progenitors that give rise to the monocyte/macrophage lineage. The precursor cells of osteoclasts are recruited to the bone surface, where they fuse to produce a large multinucleated cell, which is the mature osteoclasts. The differentiation of osteoclast precursors into mature osteoclasts is mediated by RANKL, which is produced as a result of the interaction between osteoclast precursors and stromal cells and osteoblasts. The differentiation process is influenced by various intermediary factors including PTH, Vitamin-D, IL-6, IL-11, and PGE2. The central mediator of osteoclast differentiation, RANKL, plays a crucial role in the regulation of bone resorption and bone remodeling [64]. RANKL is an essential element for osteoclastogenesis and is expressed by various cell types such as osteoblasts, osteocytes, and stromal cells. Receptor activator of NFκB (RANK) is a membrane-bound TNF receptor family member constituent that is expressed on the surface of osteoclasts. The binding of RANKL to its receptor RANK on osteoclast precursors triggers the induction of osteoclast formation. Due to the absence of osteoclasts, RANKL/RANK null rodents develop severe osteopetrosis [65,66]. Another member of the TNF superfamily called osteoprotegerin (OPG), which is produced by severalcells, including gingival and periodontal fibroblasts, osteoblasts, and stromal cells [64,67]. OPG is a soluble factor that functions as a decoy receptor for RANKL. It acts by binding to RANKL and prevents the RANK/RANKL interaction and, ultimately, inhibiting the osteoclastogenesis [64]. Consequently, the RANKL/RANK/OPG system is a vital mediator of osteoclastogenesis. Due to an increase in the number of osteoclasts, rodents lacking OPG develop osteoporosis.

Osteoblasts are the bone-forming cells. They are derived from mesenchymal stem cells, which are pluripotent cells that can differentiate into numerous cell types, such as myoblasts, adipocytes, chondrocytes and osteoblasts. The primary function of osteoblasts is to produce an organic matrix that contains numerous growth factors, which are essential for bone growth and repair. The differentiation of osteoblasts occurs through the commitment of mesenchymal precursors to osteoprogenitor lineages, which is facilitated by the sequential action of transcription factors, leading to the terminal differentiation of osteocytes [68]. The commitment of MSC to the osteoprogenitor ancestry requires the expression of particular genes. For the advancement and differentiation of osteoblasts, the transcription factors Runx2 and Osterix are irreplaceable. The regulatory activity of these pivotal osteoblast regulators is modulated by cofactors, which include members of the Dlx (distaless), Msx, and Hox homeodomain gene families, and downstream signal transduction mediators, like TGFβ superfamily-related SMADs. Osteoblasts produce organic bone matrix (osteoid), first by secreting mainly type I collagen and non-collagenous proteins like osteopontin and osteocalcin, and proteoglycans, thereafter mineralization of the organic matrix will take place. Osteoblasts become embedded in their own product, which is termed an osteocyte at this stage.

Osteocytes constitute as much as 90-95% of all total bone cells and are the most abundant and long-lived cells. Osteocytes generate an interconnected network in the bone that enables intercellular communication among themselves and the surface-lining osteoblasts [69]. For a long time, it was believed that these cells are passive cells, and their functions were misinterpreted probably due to difficulties in isolating osteocytes from bone matrix. Osteocytes are now known to sense mechanical load via their canalicular processes and instigate a series of biochemical signaling events that coordinate and influence the activity of osteoprogenitor cells, osteoblasts, and osteoclasts, which remodel bone mass in response [70,71]. The anabolic response of bone to mechanical stimuli is influenced significantly by the existence of Sclerostin. The presence of mechanical loading inhibits the expression of Sclerostin mRNA and protein, thereby reducing the suppression of new bone formation [72]. Adult bone is perpetually remodeled by the coordinated activities of bone-resorbing osteoclasts and bone-forming osteoblasts [73]. Continuous remodeling is an essential element to substitute defective bone, mend fractures, and release calcium which is essential for multiple metabolic processes. The balance between the activities of osteoclast and osteoblast is what sustains the stable bone mass (Figure 1). Disruption of the balance between resorption by osteoclasts and bone formation by osteoblasts can lead to pathological conditions such as osteoporosis or osteopetrosis. An excess of resorption without corresponding formation of new bone results in bone loss and osteoporosis, while the opposite may result in osteopetrosis [16]. It is therefore crucial to maintain this equilibrium in order to avoid serious pathological outcomes [74-76].

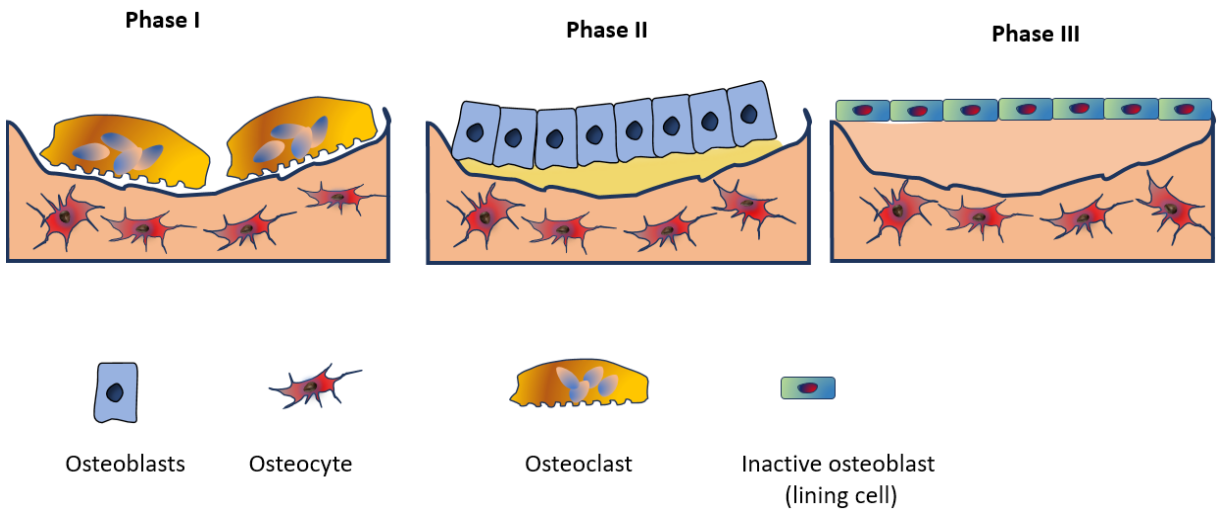

Figure 1: The Bone Remodeling Cycle consists of three phases. First, mature osteoclasts resorb damaged bone (phase I). In the second phase, mature osteoblasts produce a new bone matrix that is not yet mineralized, also known as osteoid bone. Finally, in phase III, the osteoid bone becomes mineralized, and the active osteoblasts become inactive or lining cells.

TGFB and Bone

Bone is an attractive location for cancer metastases due to the involvement of growth factors, cytokines, and cell adhesion molecules in the bone remodeling process. TGFβ1 is one of the most prevalent bone matrix growth factors [77]. It is necessary for bone remodeling and can influence the process of bone formation and bone resorption. The effects of TGFβ on osteoblasts, osteoclasts, and bone remodeling are spatially and temporally complex and variable [78]. Once osteoclasts have resorbed the bone, there follows a period of reversal, after which osteoblasts will deposit fresh bone matrix to fill the resorption cavity, known as coupling. The newly deposited collagenous matrix will be mineralized after a period of repose. Accumulating evidence suggests that TGFβ is a critical mediator in coupling bone resorption and formation [57,79]. TGFβ is embedded in the mineralized bone matrix, where it is secreted by osteoblasts [80,81]. Latent TGFβ is contained within the bone matrix. Upon bone resorption by osteoclasts, TGFβ is released and activated, which in turn stimulates the proliferation of osteoblast precursors that migrate to the resorption sites [82]. The potential for differentiation of osteoblast precursors can be facilitated through the exposure of bone mineral matrix and the release of osteotropic factors, including but not limited to BMPs, IGF-I, and PDGF [83,84]. TGFβ inhibits osteoblast differentiation and bone mineralization during the final stages of osteoblast differentiation [85]. In a coculture system of osteoclast precursors with osteoblasts and stromal cells, TGFβ causes inhibition of resorption factors like RANKL and M-CSF. Furthermore, it enhances molecules that activate the expression of osteoclast inhibitors, such as OPG [86,87].

TGFβ is a significant regulator of osteoclast function, both directly and indirectly, via its effect on osteoblasts. The significant effect of TGFβ in osteoclastogenesis is evident, but the precise mechanism remains largely unknown. During bone resorption, osteoclasts secrete cathepsins, which proteolytically liberate active TGFβ from the latent complex [88,89]. Furthermore, because osteoclasts express both TGFβ and its receptors, they are capable of directly responding to TGFβ signaling. In fetal bone culture, TGFβ was able to inhibit the recruitment of osteoclast precursors but stimulated the proliferation and differentiation of osteoclast precursors which increased bone resorption. Lee et al. reported that TGFβ1, dose-dependently decreases the number of TRAP-positive multinucleated cells after 2 and 6 days of incubation with 20 ng and 40 ng/mL M-CSF, respectively [90]. TGFβ1 inhibited RANKL expression and osteoclast-supporting activity of osteoblasts/stromal cells induced by D3 and Dex through RXR-protein degradation mediated by the ubiquitin-proteasome system. TGFβ1 exerts complex and biphasic effects on osteoclastogenesis [91]. TGFβ also increases osteoblast lineage RANKL expression, thereby fostering the recruitment of osteoclast precursors [92].

Recent studies carried out by Nguyen et al. have demonstrated that the TGFβ pathway's net activity in osteocytes is rapidly impeded by mechanical load. This reduction in Smad2 and Smad3 phosphorylation and activity undermines the bone's anabolic response to mechanical load, thus highlighting the crucial role that mechanosensitive regulation of TGFβ signaling plays in mechanical load-induced bone formation. These findings have significant implications for the development of therapies aimed at enhancing bone formation and improving bone health

in individuals with osteoporosis or other bone-related disorders [93]. Our study, which employed pharmacologic TGFβ receptor type I kinase inhibitors or a genetic model of osteocyte-specific TGFβ receptor ablation, demonstrated that the suppression of TGFβ signaling leads to the severe deterioration of the osteocyte canalicular network and dysregulates the expression of a significant number of PLR (perilacunar remodeling) genes. Bone matrix mineralization is diminished in the absence of osteocyte-intrinsic TGFβ signaling. Due to the fact that TbRIIocy/cortical bone mass and geometry are normal, the extreme fragility of these bones demonstrates that TGFβ regulates bone quality through an osteocyte-intrinsic mechanism that is dependent on PLR [94].

TGF-B and Bone Metastases

Bone is a prevalent location for cancer metastasis in several solid tumors, including breast, prostate, and lung. The microenvironment of bone contains a large amount of several growth factors, among which TGFβ is predominantly present. The metaphyseal bone, which is composed predominantly of trabecular bone and exhibits a high degree of vascularity, appears to be the most prevalent site for bone metastases. It has been observed that approximately 70% of patients afflicted with metastatic breast cancer develop bone metastases. This can lead to a variety of debilitating skeletal-related events that greatly reduce the patient's quality of life, such as pain, fractures, nerve compression, and hypercalcemia. These complications typically arise as a delayed complication of the cancer, and can have a profound impact on the patient's well-being [5,6].

Metastasis to the bone is a complicated and multifaceted process that requires the interaction of tumor and host cells. It involves the dissemination of tumor cells, which enter the circulation and travel to distal bone sinusoids. Upon reaching the bone marrow, the cells extravasate and initiate the process of bone colonization. Once colonizing the bone marrow, they start growing into macrometastases [95]. Seventy-five percent of breast cancer bone metastasis samples display affirmative nuclear staining for phosphorylated-Smad2, as observed through histological sections, thereby indicating active TGFβ signaling [96]. TGFβ signaling pathway plays a pivotal role in the development of bone metastases. The function and usefulness of TGFβ have been shown to be complex and context-dependent in many studies. Breast cancer cell line MDA-MB-231 cells were transduced with a retroviral vector expressing a reporter gene under the control of a TGFβ–sensitive promoter in an animal model of breast cancer bone metastases. It was demonstrated in this experiment that the use of this reporter identified active TGFβ-Smad signaling specifically in the bone and that knocking down Smad4 expression in breast cancer cells reduced the growth of bone metastases [96]. The findings of an alternate bone metastases model indicate that the inhibitory Smad7 expression significantly reduced bone metastases in 1205Lu melanoma models, providing further evidence of the contribution of TGFβ in this process [97].

TGFβ can promote and exacerbate bone metastases by inducing specific genes. Osteolytic bone destruction is the outcome when cancer cells with bone metastasis produce factors that stimulate osteoclast activity and causes bone destruction. Such factors include Parathyroid Hormone-Related Protein (PTHrP) and interleukin 11 (IL11) [88]. Upon osteoclastic bone resorption, TGFβ is released from the mineralized bone matrix and activated. This will further enhance the production of pro-osteolytic factors, including Parathyroid Hormone-Related Protein (PTHrP), interleukin 11 (IL11), Connective Tissue Growth Factor (CTGF), and matrix metalloproteinase-1 (MMP-1), CXCR4, and [98,99] (Figure 2). The signaling pathway of TGFβ-Smad is known to stimulate the expression of PTHrP, which is extensively expressed in various tissues and has a similar sequence with PTH. PTHrP is also expressed in the majority of breast cancer and bone metastases, playing a significant role in the development of osteolytic lesions and causing the humoral hypercalcemia of malignancy [100]. According to a prospective study, the expression of PTHrP in primary breast cancer was substantially associated with fewer bone metastases [101-103]. This particular research endeavor potentially offers a rationale for the observed escalation in PTHrP expression within breast cancer bone metastases. This phenomenon arises due to the discharge of TGFβ from the mineralized bone matrix that follows bone resorption, rather than the intrinsic amplification of PTHrP expression in tumor cells that colonized the bone. In fact, inhibiting TGFβ signaling in MDA-231 breast cancer cells through stable transfection of a dominant negative TRII (DNTRII) led to the inhibition of TGFβ-induced expression of PTHrP production in tumor cells. Consequently, this inhibition curtailed the onset of osteolytic lesions. Yin et al. were the pioneers in demonstrating this in a model of bone metastases in mice [13]. In MDA-MB-231 breast cancer cells, stable overexpression of dominant-negative Smad 2, 3, and 4 decreased PTHrP production, according to another study. This inhibited the stimulated production of RANKL, which [104], This inhibited the stimulated production of RANKL, which induces osteoclast differentiation and activation and fosters bone metastasis [105]. This inhibited the stimulated production of RANKL, which induces osteoclast differentiation and activation and fosters bone metastasis. Both IL-11 and CTGF are considered osteolytic genes. In osteoblasts, IL-11 stimulates the expression of osteoclastogenic factors RANKL and GM-CSF, thereby promoting bone resorption. CTGF is an extracellular invasion and angiogenesis mediator. In metastatic cells, both IL-11 and CTGF are directly regulated by TGFβ via the canonical TGFβ /Smad pathway [12].

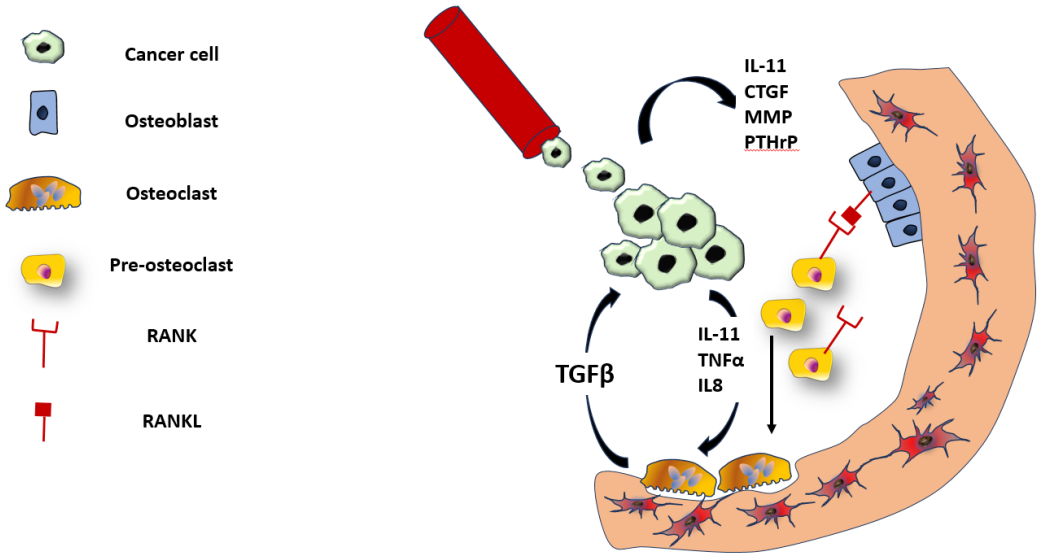

Figure 2: Shows that cancer cells spread through the bloodstream and settle in the bones. These cancer cells trigger the breakdown of bone tissue by directly stimulating osteoclasts through the release of osteolytic factors, or indirectly b y the release of certain factors that can stimulate osteoblasts to express RANKL, which then binds to RANK receptors on preosteoclasts. This can lead to an increase in bone resorption and the release of TGFβ from the bone matrix, creating a cycle that perpetuates itself.

Hypoxia is a phenomenon characterized by oxygen deficiency, and is observed in a significant proportion of solid tumors [106]. The regulation of transcription by hypoxia-inducible factor 1 (HIF-1) is a main mechanism mediating adaptive hypoxia. The bone microenvironment is considered hypoxic, with oxygen levels ranging between 1 and 7 percent [106]. During the progression of cancer, the expression and activation of hypoxia-inducible factors (HIFs) are frequently observed. This is usually associated with the acquisition of a more malignant phenotype. Hypoxic cells are also believed to be resistant to most of anticancer drugs in part due to the upregulation of genes linked to drug resistance [107-109]. As a result, developing new strategies to target hypoxic cells holds great potential for improving outcomes in cancer patients.

HIF-1 has been shown to promote the formation of osteolytic bone metastases from MDA-MB-231 breast cancer cells by stimulating angiogenesis, osteoclastogenesis and inhibiting the differentiation of osteoblasts [110]. There are numerous interactions between hypoxia and TGFβ biology. TGFβ inhibits the degradation of HIF-1a, resulting in its stabilization. According to in vitro data [111,112], HIF-1 and TGFβ -induce vascular endothelial growth factor (VEGF) and CXCR4 with additive effects. In a mouse model of breast cancer bone metastases, inhibition of HIF-1 or TGFβ by knockdown or DNTRII reduces metastasis formation significantly without additive effect [112]. When both cancer cells and the bone microenvironment were targeted with a combined pharmacological inhibition of HIF-1 and TGFβ, they exhibited a more significant additive effect than single treatment. The findings suggest that bone metastases are driven by both hypoxia and TGFβ signaling concurrently, and that the use of pharmacological inhibitors targeting both tumor cells and the bone microenvironment can effectively reduce tumor burden in an additive manner [112].

EZH2 is a histone methyltransferase that functions as an enzymatic subunit of the polycomb repressive complex 2. A positive correlation exists between overexpression of EZH2 and metastasis of solid tumors, such as prostate and breast cancers. Furthermore, in women with early-stage hereditary breast cancer, EZH2 is considered a prognostic biomarker of risk development of metastases risk [113,114]. Zhang et al. found that depletion of EZH2 inhibited breast cancer bone metastasis in vivo. This inhibition is accomplished by EZH2's ability to increase the transcription of integrin 1-encoding TGβ1, which, in turn, activates focal adhesion kinase (FAK), a downstream effector. FAK activation phosphorylates TβRI and increases its binding to TβRII, thereby activating the TGFβ signaling pathway. This study revealed a cooperative relationship between EZH2 and TGFβ signaling in breast cancer bone metastasis promotion through the methyltransferase-independent pathway [115]. Numerous studies have shown that long noncoding RNAs (lncRNAs) play crucial roles in the preponderance of cellular processes, such as growth, invasion, migration, and stemness. Dysregulated expression of lncRNA has been reported in association with tumor initiation

and metastasis in numerous human malignancies and metastases [116,117]. A prostate cancer (PCa)-associated, long noncoding RNA (lncRNA), prostate cancer-associated transcript 7 (PCAT7), also known as PCAN-R2 is an RNA located in chromosome (chr) 9q22.32 and has been found to play a role in tumor progression [118]. Lang et al., demonstrated that primary PCa tissues with bone metastasis exhibited an increase in PCAT7 expression. They further found that SMAD3/SP1 transcriptional complex-induced overexpression of PCAT7 and this upregulated TβR1 expression by sponging miR324-5p as a ceRNA. Consequently, this led to the unrestrained activation of TGFβ pathway, which in turn promoted prostate cancer bone metastasis [119]. Long noncoding RNA small nucleolar RNA host gene 3 (SNHG3) has been implicated in the initiation and progression of multiple human cancers [120]. Xi et al., showed that PCa tissues with bone metastasis had higher SNHG3 expression than PCa tissues without bone metastasis. A statistically significant link was found between high levels of SNHG3 expression and advanced clinical features, bone metastasis, and a poor prognosis in prostate cancer patients. In vitro silencing of SNHG3 inhibited PCa cell proliferation and metastatic behavior. In addition, SNHG3 suppression inhibited PCa cell bone metastasis in vivo. Through activating TGFβ signaling, SNHG3 acted as a reservoir for miR-214-3p to increase TGFβR1 expression, which in turn promoted the growth of the primary tumor and bone metastasis in prostate cancer [121].

In various stages of bone metastasis, a complex interaction between TGFβ and Wnt signaling enables efficient colonization, dormancy, and outgrowth. While TGFβ signal is responsible for preserving the quiescence of Disseminated Tumor Cells (DTCs) in bone [122], Wnt inhibition by DKK1 is essential for immune evasion by quiescent micrometastases [123] and late-stage osteoclastogenesis [124]. On the other hand, DTCs' engagement with bone vascular E-selectin promotes metastatic colonization by activating Wnt signaling. Esposito et al. recently discovered that TGFβ induces DACT1 protein condenses in the cytoplasm to inhibit Wnt signaling, and the DACT1 condensates are maintained in vivo. Furthermore, the study showed that DACT1 is crucial for breast and prostate cancer bone metastases. This research highlights the delicate interplay between TGFβ and Wnt signaling pathways in bone metastases and the potential for targeted interventions in cancer treatment [125].

TGF-B as Therapeutic Target in Cancer and Bone Metastases

The TGFβ signaling pathway has a wide range of effects, which makes it a promising target for therapeutic interventions in disease treatment. There are three major categories of TGFβ inhibitors that have been extensively studied. : [1] The first category is receptor kinase inhibitors, which work by inhibiting the kinase activity of TRI/ALK5, along with TRII, to prevent downstream signaling; and [3] The second category is ligand traps, which are composed of monoclonal neutralizing TGFβ antibodies and soluble decoy receptor proteins; [2] Lastly, antisense oligonucleotides fall under the third category, as they function by blocking TGFβ expression at the transcriptional/translational level.

Small Molecule Receptor Kinase Inhibitors

TGFβ receptor kinase inhibitors are a class of small molecule inhibitors that inhibit the kinase catalytic activity of TRI/ALK5 via ATP-competitive inhibition. The development and scalability of small molecule inhibitors possess several advantages; however, the lack of selectivity associated with kinase inhibitors can be problematic. At present, all known small molecule TR1/ALK5 inhibitors [126-130] inhibit ALK4 kinase activity with the same potency as ALK7 kinase activity. SB-431542 (GlaxoSmithKline), [129] Ki26894 (Kirin Brewery Company) [131], LY364947 (Eli Lilly & Co.), and SD-208 and SD-092 (Scios Inc) are among the extensively investigated TRI/ALK5 inhibitors. Each of these substances inhibits receptor kinase activity and tumor cell proliferation, invasion, and metastasis in animal models [127-129]. SD-208 treatment considerably inhibited osteolytic lesion areas, bone metastatic growth, and improved survival in a xenograft model of intracardiac-inoculation of MDA-MB-231 human breast cancer cells. Furthermore, treatment with SD-208 inhibited further tumor growth and the formation of osteolytic lesions in rodents with established bone metastases [112,132]. The same treatment has been shown to increase bone mass in non-tumor models, which may have reciprocal benefits for cancer patients (reduction of osteolytic lesions and increase in bone mass) [133]. LY364947 inhibited TGFβ-induced Smad2 phosphorylation, MDA-MB-231 breast cancer cell invasion and fibronectin expression [134]. LY364947 decreased cell proliferation and ATP production in a concentration-dependent manner in the 2D Model of ovarian cancer cell lines [135]. SB-431542 functions as a potent inhibitor of Smad3 phosphorylation. This compound is highly selective and can specifically target endogenous TGFβ and activin. Importantly, it has no significant impact on BMP signaling, nor does it possess the ability to inhibit other serine-threonine kinases, including components of the ERK, JNK, or p38 MAP kinase pathways. These findings suggest that SB-431542 represents a promising pharmacological tool for investigating the role of Smad3 phosphorylation in various biological processes [126, 136].

Neutralizing Antibodies and Soluble Decoy Receptor Proteins

TGFβ1 is the most frequently expressed isoform in various human tumors. TGFβ levels and downstream signaling are frequently elevated during the progression of cancer and are associated with tumor aggressiveness and grade/stage

[122,123,137]. Either TGFβ ligand trap, which employs a soluble decoy receptor composed of the TRII or TRIII ectodomain, or TGFβ neutralizing antibodies can reduce the amount of active TGFβ signaling. Individual ligands and all three isomers of TGFβ have been targeted by neutralizing antibodies (pan-neutralizing antibodies). It appears that specific TGFβ1 inhibition could provide the desired antitumor effects without the cardiovascular toxicity associated with the use of either TGFβ2 or TGFβ3 inhibition. However, all three isoforms use the same receptor, so using small molecule inhibitors of the receptor's kinase activity cannot achieve this. This, however, is feasible with isoform-specific neutralizing antibodies, and a recent report demonstrated the anti-tumor efficacy of a novel TGFβ1-specific mAb administered in combination with anti- PD1 checkpoint inhibitors in preclinical tumor models [138]. A report suggested that TGFβ1 blockade could surmount the TGFβ signature-associated primary resistance of human malignancies to checkpoint inhibitors [139,140]. It is unknown whether TGFβ1-specific inhibition can also exert antitumor effects in conjunction with other immunotherapies or independently of immunotherapy. In mouse tumor models, the pan-neutralizing mouse monoclonal antibodies 1D11 and 2G7 bind and inhibit the biological activity of all three TGFβ isoforms, demonstrating their therapeutic potential. The treatment of mice harboring MDA-MB-231 breast cancer cells and PC3 prostate cancer cell completely abrogated tumor growth [132,141] and inhibited the growth of established MDA-MB-231 subcutaneous tumors and pulmonary metastases in athymic mice [142]. Similarly, administration of 1D 11 to rodents injected orthotopically with 4T1 breast cancer cells inhibited lung metastasis [143-145]. 1D11 has also been shown to reduce skeletal tumor burden and osteolytic bone lesions caused by MDA-MB-231 cells [146].

Using recombinant Fc-fusion proteins containing the soluble ectodomains of TRII or TRIII is an additional method for inhibiting the binding of TGF to its receptors. In animal models, it has been demonstrated that these biologically active compounds inhibit lung and breast cancer metastasis [43,147-149]. Cane et al. reported a synergistic effect of anti-TGFβ-1 and a preventative cancer vaccine in the CT26 colon carcinoma model of preclinical animal studies. In an autochthonous model of melanoma, the anti-TGFβ-1 antibody also demonstrated therapeutic efficacy as a monotherapy, preventing tumor progression by inhibiting EMT induction. These findings encourage the continued development of anti-TGFβ1 antibodies for use in a variety of cancer treatment settings [150].

Antisense Oligonucleotides

Antisense Oligonucleotides (ASOs) inhibit the expression of a specific target protein. ASOs are 13-25 nucleotide single-stranded polynucleotide compounds designed to hybridize with complementary RNA sequences. ASOs inhibit mRNA function and protein synthesis through splicing modulation and translation inhibition [151,152]. ASOs against TGFβ decrease the bioavailability of active ligands in the microenvironment of the local tumor. Muraoka-Cook et al. [153] employed an orthotopic model of PyMT mammary tumors to examine the role of autocrine TGFβ in metastasis formation. Overexpression of a TGFβ ASO decreased metastasis and survival [153], whereas PyMT tumors overexpressing TGFβ increased metastasis and survival. A melanoma-bearing humanized mouse model study examined the complementary effects of transforming growth factor-β2 (TGFβ2) anti-sense oligodeoxynucleotide (TASO). The combination of TASO and IL-2 was found to promote the infiltration of CTLs into the tumor, thereby augmenting the tumor-killing function of Cytotoxic T Lymphocytes (CTLs) in conjunction with elevated granzyme B expression. Additionally, TASO has been shown to abate the rise of Tregs in the peripheral blood and spleen that is typically triggered by IL-2. The compound also curbs the infiltration of Tregs into tumors, a phenomenon that can be attributed in part to the lowered production of CCL22. Inhibition of TGFβ2 has been proposed as a mechanism for the modification of T-cell constituents at the periphery when used together with IL-2, which may be linked to a synergistic upregulation of serum pro-inflammatory cytokines and a decrease in the ratio of Tregs to CTLs in tumor tissues. This ultimately leads to a marked suppression of tumor growth [154]. Toll-like receptor 9 (TLR9) agonist CpG ODN and Transforming growth factor-2 (TGFβ2) antisense oligodeoxynucleotide TIO3 were given to mice in order to induce the formation of TME, which led to the enrichment and activation of CD8+ T cells and NK cells. This was accompanied by a significant decrease in TGFβ2. The combination treatment not only significantly slowed the progression of the cancer in the mice but also increased their chances of surviving the disease. It also shielded the tumor-free animals against a subsequent attack by the cancer [155]. OT-101 (trabedersen) is an 18-mer phosphorothioate antisense oligodeoxynucleotide designed to specifically target the human TGFβ2 mRNA. TGFβ2 isoforms are shown to be overexpressed in tumor tissue and plasma of Pancreatic Ductal Carcinoma (PAC) and other cancers [156]. OT-101 inhibited TGFβ2 secretion in PAC cell lines (Hup-T3, Hup-T4, and PA-TU-8902). Additionally, it rapidly slowed cell proliferation and totally stopped the migration of PAC cells. Most significantly, when given at dosages suitable for therapeutic use, OT-101 reversed the Lymphokine-Activated Killer (LAK) cell-targeted PAC cells' TGFβ2-mediated immunosuppression, which significantly increased the cytotoxicity of LAK cell-mediated killing [157,158].

Other Molecules That Antagonize TGFβ

Further biologically active molecules that inhibit TGFβ or its signaling have been identified. Halofuginone (HF), a derivative

of a natural product, is an alkaloid originally isolated from the Chinese plant Dichroa febrifuga. It has been approved by the US FDA for the prevention of coccidiosis in poultry [159]. In vitro experiments have demonstrated that HF inhibits TGFβ signaling in various cell types. Furthermore, the daily administration of HF to mice has been found to significantly impede the formation of bone osteolytic lesions and bone metastases following the intracardiac injection of melanoma 1205Lu cells [160]. HF has been shown to induce apoptosis of breast cancer cells and inhibited cell migration via downregulation of Matrix Metalloproteinase-9 (MMP-9) [161]. In a mouse model of breast and prostate cancer bone metastases, we demonstrated that, HF treatment reduced bone destruction in these two models [162]. HF treatment is a novel agent that inhibits TGFβ signaling in bone metastasis, despite the fact that the precise mechanism of action remains unknown.

Combination Therapy

Combining treatments that antagonize the effects of TGFβ with other therapies is an attractive method for increasing treatment efficacy in patients with bone metastases. As was demonstrated for rapamycin [163] and doxorubicin [164,165], inhibiting TGFβ signaling can improve the therapeutic efficacy of a variety of cytotoxic agents. Based on our research, it has been found that the combination of SD-208 and a bone resorption inhibitor such as, zoledronic acid, exhibited a more effective reduction in the progression of established osteolytic breast cancer metastases than either therapy alone [166]. Using the same bone metastasis paradigm of intracardiac inoculation of MDA-MB-231 breast cancer cells, we conducted an evaluation of the combined effects of SD-208 and 2-methoxyestradiol, an inhibitor of HIF-1, the key mediator of hypoxia. The results of this evaluation indicated that the combination of these agents results in more effective reduction in osteolytic lesion areas and tumor burden as well as an improvement in mouse survival than either agent alone [112]. Immune checkpoint molecules are rising to prominence as potential targets for cancer immunotherapy due to their ability to induce long-lasting remission in patients with metastatic disease. Despite the fact that, several antibodies that target programmed death ligand 1 (PD-L1), such as atezolizumab, avelumab, and durvalumab, demonstrate spectacular and lasting remissions, these antibodies only demonstrate their effectiveness in a subset of specific cancer types [167,168]. In several ongoing preclinical and clinical trials, anti-PD-L1 monoclonal antibodies (mAb) is being evaluated in conjunction with cancer-modulating therapies or other immunostimulatory medicines with the goal of increasing the therapeutic effectiveness of the treatment [169]. In a multicenter clinical trial, a single-arm phase Ib study was conducted. It consists of a dose- finding phase (Part A) followed by an expansion cohort phase (Part B). The study was carried out at 11 sites in France, Italy, the Republic of Korea, Spain, and the USA with the aim of testing the use of the galunisertib (a selective TGFβRI inhibitor) co-administered with durvalumab (a selective, high-affinity, human IgG1 monoclonal antibody that targets PD-L1) for locally advanced or metastatic pancreatic adenocarcinoma [170] . The results of the phase 1b study indicated that no dose limiting toxicities (DLTs) were observed, establishing galunisertib 150 mg two times per day plus durvalumab 1500 mg Q4W as the recommended phase II dose (RP2D). Urthermore, no new safety issues were identified with galunisertib and durvalumab relative to either drug given as monotherapy, suggesting that this combination has an acceptable tolerability and safety profile [170].

Immune checkpoint treatment has encouraging results for patients with metastatic castration-resistant prostate cancer (CRPC), although it performs less well for bone metastases. Immune checkpoint therapy had little effect in an intraosseous mouse model of bone CRPC of syngeneic Myc-CaP prostate cancer cells, despite having a considerable impact on subcutaneous tumor growth. However, immune checkpoint treatment combined with TGFβ blockade enhanced Th1 cells, which led to a decline in bone CRPC [171]. However, it was hypothesized that PD-1 inhibition would act as a safeguard against cancer's bone-damaging effects. In a recent study, PD-$1^{-/-}$ animals demonstrated exceptional resistance to bone loss brought on by femoral inoculation of Lewis lung cancer cells [172]. In order to improve the efficacy of immunotherapy, it is essential to find ways to dampen the immune suppression that is caused by the microenvironment of the tumor.

Risks and Limitations

The potential therapeutic possibilities for treating various disease indications are highly attractive as a result of the biological importance and wide-ranging effects of TGFβ and its signaling. Nonetheless, caution must be exercised in the potent and/or prolonged blockade of this significant biomolecule, as it may lead to an array of undesirable adverse effects., hence, the development of specific TGFβ blockade is challenging. Therapies based on ASOs and large biologicals (neutralizing antibodies) must surmount obstacles in drug delivery. The development of small molecules, specifically those that target the TGFβ receptor kinase, offers a viable alternative to injectable delivery and addresses the commonly observed issues of neutralizing antibody generation and tissue penetration associated with biologic-based agents, while the majority of these molecules can be administered orally [15]. However, TGFβ receptor kinase inhibitors currently in use are less selective than TGFβ ASOs and TGFβ -directed biological therapies. The employment of bisphosphonate to achieve targeted drug delivery to bone represents a promising strategy to circumvent issues of off-target tissue toxicity and suboptimal drug exposure to tumor cells in bone metastatic disease. In this regard, the use of a bisphosphonate-coated liposome as a targeting agent may prove advantageous in sites of pronounced bone turnover, such

as those associated with metastatic bone disease. This approach appears to hold significant potential for improving the efficacy of bone-targeted therapeutic interventions [165]. Targeting bone diseases through the conjugation of small molecule inhibitors to bisphosphonates using anti-TGFβ therapies is a viable option. By utilizing these techniques that specifically target the bones, it is possible to achieve extended and concentrated exposure to the therapeutic compounds in the affected area, thereby increasing their efficacy and reducing the incidence of overall negative side effects.

Conclusion

TGFβ antibodies, TGFβ receptor kinase inhibitors, and TGFβ antisense oligonucleotides, all of which target the TGFβ pathway, have undergone assessment in preclinical animal models, as well as clinical trials in cancer patients, including those with bone metastases. Presently, the use of TGFβ targeting agents is impeded by toxicities. Combining TGFβ inhibitors with bone-targeted agents could potentially offer more favorable outcomes compared to using single agents, whilst also circumventing certain drug limitations. In this epoch of immunotherapy, novel therapeutic strategies that merge TGFβ inhibitors with PD-1/PD-L1–mediated inhibition or with cell-based therapies like CAR-T cells are being formulated with the aim of enhancing treatment outcome. Neutralizing TGFβ by using anti-TGFβ antibodies in solid malignancies with bone metastases can potentially improve the efficacy of immune checkpoint inhibition due to the high concentration of TGFβ found in the bone matrix. The positive results from preliminary clinical trials utilizing a combined therapeutic strategy of immunotherapy and targeting TGFβ should serve as motivation for further exploration of these methods to overcome the current restrictions of TGFβ pathway inhibition. At this time, the use of TGFβ-targeting agents is limited due to associated toxicities. However, the combination of TGFβ inhibitors with bone-targeted agents may provide more advantageous effects compared to single agents and potentially bypass certain drug limitations. Therapeutic approaches that combine TGFβ inhibitors with PD-1/PD-L1-mediated inhibition or with cell-based therapies, like CAR-T, are under development. Given the abundance of TGFβ in bone, neutralizing TGFβ with anti-TGFβ antibodies may augment the efficacy of immune checkpoint inhibition in solid malignancies with bone metastases. The successful results of early clinical trials utilizing a combined treatment strategy of immunotherapy and TGFβ targeting strongly encourage continued exploration of these methods to overcome the obstacles associated with inhibiting the TGFβ pathway.

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