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Review

TGF-β at the Crossroads: Orchestrating the Bone Metastatic Microenvironment and Shaping Therapeutic Frontiers

Khalid S. Mohammad[1,*], Fatimah Hussain Bu Izran[1]

[1]Department of Anatomy, College of Medicine, Alfaisal University, 11533 Riyadh, Saudi Arabia
*Correspondence: kmohammad@alfaisal.edu (Khalid S. Mohammad)

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Abstract

Bone remains one of the most hospitable—and devastating—destinations for metastatic cancer cells. At the center of this unwelcome alliance is transforming growth factor-β (TGF-β), a cytokine stored in the mineralized matrix and unleashed during osteoclastic bone resorption. Once activated, TGF-β fuels a self-reinforcing "vicious cycle": it co-opts tumor cells to undergo epithelial-to-mesenchymal transition, recruits and primes osteoclasts, suppresses osteoblast function, and shapes an immunosuppressive niche that shields malignant clones. The result is a micro-environment exquisitely tuned for tumor survival, skeletal destruction, and therapy resistance. This review traces the molecular choreography of TGF-β signaling within the bone tumor microenvironment (TME), detailing its crosstalk with osteogenic, immune, and stromal compartments across breast, prostate, and lung cancer metastases. We synthesize pre-clinical and clinical efforts to interrupt this pathway, ranging from ligand-neutralizing antibodies and activin receptor-like kinase 5 (ALK5) kinase inhibitors to antisense oligonucleotides and tumor-selective ligand traps—and examine why benefits observed in early trials are tempered by dose-limiting toxicities and adaptive resistance. Beyond TGF-β itself, we highlight parallel targets in the TME, including receptor activator of nuclear factor kappa-B ligand (RANKL)-driven osteoclastogenesis, vascular endothelial growth factor/fibroblast growth factor (VEGF/FGF)-mediated angiogenesis, and immune checkpoints such as PD-1, TIM-3, and LAG-3, arguing that multi-pronged combinations guided by real-time TME profiling offer the most promising path forward. We outline pressing research priorities: mapping the spatiotemporal dynamics of TGF-β activation, identifying predictive biomarkers for patient stratification, and engineering bone-targeted delivery systems that preserve normal tissue repair. By decoding and disrupting the TGF-β-centered circuitry of bone metastasis, we can move closer to therapies that not only palliate skeletal complications but also prolong life for patients with advanced cancer.

Keywords: TGFβ signaling; bone metastasis; tumor microenvironment; epithelial–mesenchymal transition; targeted therapy

1. Introduction

Bone metastases represent a significant clinical concern across various cancer types, notably breast, prostate, and lung cancers, due to their high incidence, prevalence, and associated morbidity and mortality. The skeletal system is a common site for metastasis due to its rich blood supply and supportive microenvironment, which facilitates the colonization of tumor cells. Understanding these dynamics informs clinical management strategies and provides insights into patient prognosis, particularly regarding skeletal-related events (SREs) that stem from these metastases.

1.1 Incidence and Prevalence of Bone Metastases

Bone metastases are reported to occur in a considerable percentage of patients suffering from primary cancers. Studies indicate that approximately 68% of metastatic bone diseases arise from three major cancers: breast, prostate, and lung cancer [1]. Breast cancer is frequently noted as the leading cause of bone metastases in women, while prostate cancer holds a similar position in male patients [2]. Prostate cancer metastasizes to the bone in about 70–90% of patients at the time of advanced disease onset, rendering it one of the most prevalent forms of skeletal metastases [3,4]. Lung cancer patients exhibit notable rates of skeletal involvement, with bone metastases affecting about 30–40% of those diagnosed with advanced stages of the disease [5,6]. A recent population-based SEER analysis of $>$1 million adults diagnosed with solid tumors between 2010 and 2020 identified 55,903 patients who already had bone metastases at the time of first presentation [3,5]. Among that bone-metastatic cohort, the primary-site breakdown was lung 44.4%, prostate 19.3%, breast 12.3%, kidney 4.0%, colorectal 2.2%, pancreas 2.2%, with every other histology individually $\leq$2% [7]. In a recent study by Özdemir *et al.* [8], which focused specifically on the relative distribution among the three major primary cancers, the study found that, among metastatic lesions identified in the bone, approximately 58.0% were due to lung cancer, 29.0% were attributable to breast cancer, and 13.9% to prostate cancer. This study illustrates that while breast and prostate cancers are often highlighted due to their high propensity for bone involvement (especially in women and men, respectively), lung cancer may represent the majority of cases in some cohorts [8]. Despite variations due to ethnic and regional differences, the overall trend shows that 30% to

70% of all cancer patients may develop skeletal metastases at some point during their illness [3,9]. The prevalence of these events is not limited to advanced cases; a significant proportion of patients present with bone metastases, even at initial diagnosis, emphasizing the necessity for vigilant screening protocols [1,10]. Importantly, methodologies such as whole-body MRI have enhanced detection rates and visualization of metastatic spread, fostering improved diagnosis [11].

1.2 Skeletal-Related Events (SREs)

The morbidity associated with bone metastases primarily manifests through skeletal-related events (SREs), which encompass a spectrum of complications, including pathologic fractures, pain, and hypercalcemia. SREs significantly deteriorate the quality of life and elevate healthcare costs due to increased management requirements such as palliative care, surgical interventions, or radiotherapy [5,10,12]. Pathologic fractures are prevalent in this patient population; they occur in 7–35% of patients with lung cancer and account for a significant reason for hospitalization among those with bone metastases from breast and prostate cancers [5,13]. Hypercalcemia, often considered a paraneoplastic syndrome, emerges in 1–20% of patients and is associated with a poor prognosis due to its debilitating symptoms, including nausea, confusion, and renal dysfunction [5,12]. Overall, SREs like these not only exacerbate the patient's clinical condition but also significantly contribute to increased mortality rates, emphasizing the need for effective preventive strategies and therapeutic interventions [14,15].

1.3 Prognostic Implications

The prognosis for patients with bone metastases is generally poor, particularly when considering the burden of SREs. The presence of skeletal involvement has been correlated with reduced overall survival across multiple cancer types, with prostate cancer patients displaying an approximately 70–90% probability of developing bone involvement as the disease progresses [3,16]. SREs are directly linked to an increase in mortality; data reveal that patients with skeletal metastases experience a markedly escalated risk of dying compared to their counterparts without such complications [14,15]. Consequently, the early identification of bone metastases and the implementation of appropriate interventions are critical for improving outcomes in affected individuals.

1.4 Management Strategies

Management of bone metastases, particularly in prostate cancer, may involve bisphosphonates or denosumab, agents that can ameliorate the incidence of SREs while potentially improving quality of life [17,18]. Recent studies propose novel approaches, including targeted therapies and the utilization of radiopharmaceuticals, which have shown promise in managing advanced prostate and breast cancers alongside their metastatic complications [12,19]. The integration of palliative care from the point of diagnosis is also crucial to managing symptoms and improving patient-centered outcomes, highlighting the need for a multidisciplinary approach in treating patients with metastatic disease [12,20,21].

2. Transforming Growth Factor-beta (TGF-β) and Its Pivotal Role in Health and Disease

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that plays pivotal roles in various biological processes, including cellular growth, differentiation, apoptosis, and the immune response. As a member of the TGF-β superfamily, it is produced in various tissues, where it exerts significant effects on tissue homeostasis, repair, and fibrosis. TGF-β exists in three isoforms in humans: TGF-β1, TGF-β2, and TGF-β3. Each isoform plays distinct roles in health and disease, particularly in cancer progression and metastasis.

2.1 TGF-β Signaling

The signaling mechanisms of TGF-β are classically divided into two major categories: the canonical (Smad-dependent) and the noncanonical (Smad-independent) pathways (Fig. 1). In the canonical pathway, TGF-β binds to a heterotetrameric receptor complex composed of type I and type II serine/threonine kinase receptors. Upon ligand binding, the type II receptor phosphorylates the type I receptor, which in turn phosphorylates receptor-regulated Smads (R-Smads), typically Smad2 and Smad3 [22]. These phosphorylated R-Smads then form a complex with the common mediator Smad, Smad4, and translocate into the nucleus where they regulate the transcription of target genes involved in processes such as extracellular matrix (ECM) production and fibrotic responses [22,23]. This canonical pathway is essential in driving the transcriptional programs that underlie many of the cytokine's effects in both development and disease [24].

TGF-β can activate several noncanonical signaling cascades that do not rely primarily on Smad proteins. These include the activation of mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, as well as pathways involving phosphatidylinositol-3-kinase, protein kinase B (PI3K)/Akt and Nuclear factor-kappa B (NF-κB) signaling [24]. The noncanonical pathways provide an additional level of regulation and allow TGF-β to modulate diverse cellular responses, sometimes in a context-dependent manner that is critical for the progression of fibrotic and oncogenic processes [25,26]. For example, in several fibrotic disorders, TGF-β promotes excessive extracellular matrix (ECM) deposition through both the canonical Smad signaling and complementary noncanoni-

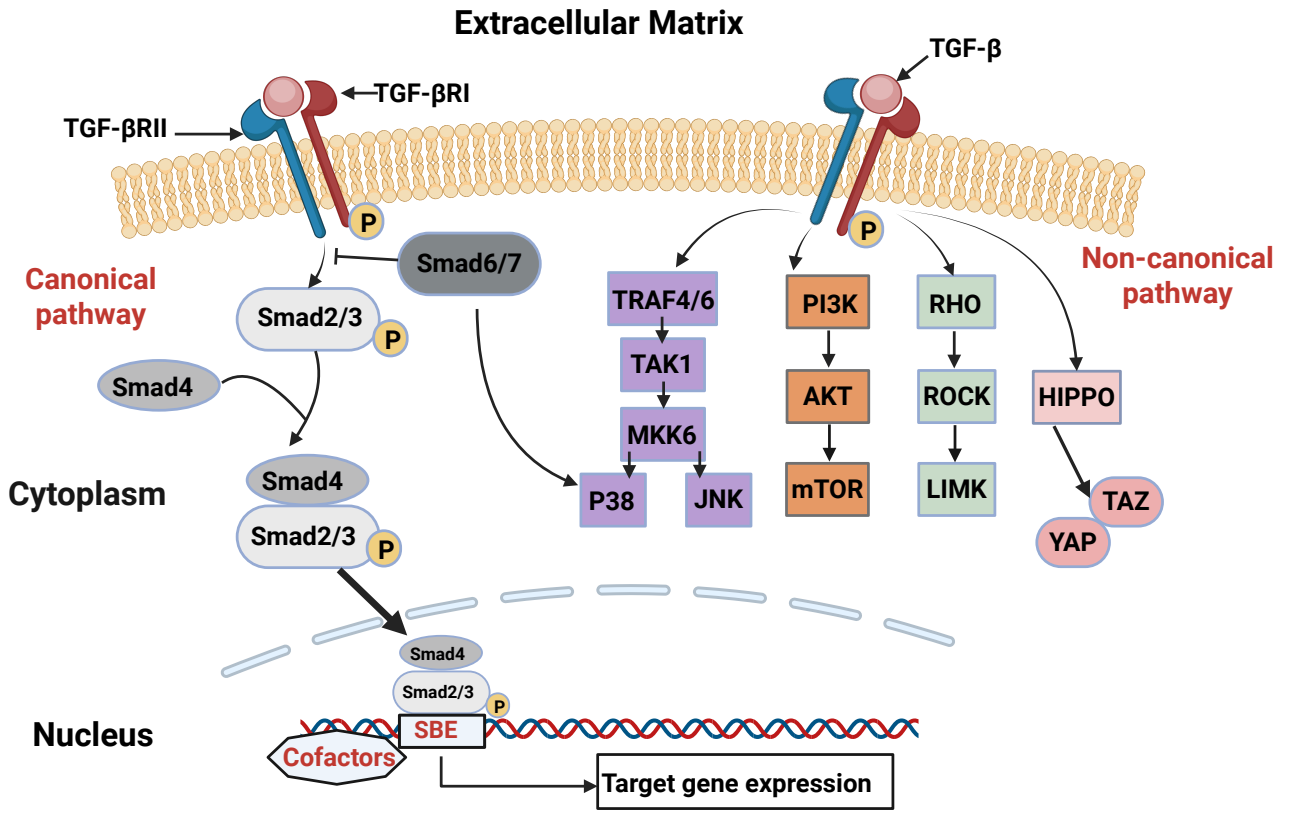

Fig. 1. Canonical and non-canonical transforming growth factor-beta (TGF-β) signaling pathways. This figure illustrates the dual branches of TGF-β signaling: the canonical (Smad-dependent) and non-canonical (Smad-independent) pathways. TGF-β ligand binding initiates signaling by forming a heteromeric complex between TGF-β receptor type II (TGF-βRII) and type I (TGF-βRI). In the canonical pathway, activated TGF-βRI phosphorylates receptor-regulated Smads (Smad2 and Smad3), which then associate with the common mediator Smad4. This complex translocates into the nucleus where, in cooperation with transcriptional cofactors, it regulates target gene expression. Negative feedback is mediated by inhibitory Smads (Smad6/7), which interfere with receptor activation or Smad complex formation. The non-canonical pathway is activated in parallel through TGF-β-induced recruitment of TNF Receptor–Associated Factor 4/6 (TRAF4/6) and activation of TGF-β-activated kinase 1 (TAK1). This cascade then activates various downstream effectors, including the MKK6-p38 and c-Jun N-terminal Kinase Mitogen-Activated Protein (JNK MAP) kinase pathways, the Phosphoinositide 3-Kinase-Protein Kinase B (PI3K-AKT-mTOR) axis, Ras Homolog Family Member A/Rho-Associated Coiled-Coil Containing Protein Kinase (RhoA/ROCK) signaling, and the Hippo pathway effectors Transcriptional Coactivator with PDZ-Binding Motif (TAZ) and Yes-Associated Protein (YAP). Collectively, these pathways regulate cytoskeletal dynamics, survival, migration, and extracellular matrix remodeling, often in a context-dependent manner. The integration of canonical and non-canonical TGF-β signals allows for precise modulation of cellular fate and behavior in both physiological and pathological settings, including cancer progression and metastasis. Created in BioRender. Mohammad, K. (2025) https://BioRender.com/k8448f9.

cal routes that further enhance cellular activation and survival [25,26].

The interplay between these signaling arms enables TGF-β to serve as a central orchestrator of cellular behavior. Its dual mode of signaling through both Smad-dependent and independent mechanisms ensures that it can integrate various intracellular cues and external stimuli to exert tightly regulated, context-specific effects in both normal physiology and disease pathology [24]. This complexity is evident in diseases such as liver fibrosis, aortic aneurysms, and cancer, where altered TGF-β signaling contributes significantly to disease progression via dysregulated canonical and noncanonical signaling [22,27].

2.2 Role of TGF-β in Health and Disease

In healthy tissue, TGF-β regulates numerous cellular mechanisms crucial for maintaining homeostasis and facilitating tissue repair. It is essential for embryonic development, wound healing, and the regulation of the ECM [28]. TGF-β promotes the differentiation of mesenchymal stem cells into chondrocytes and osteoblasts, therefore playing a vital role in bone development and homeostasis [29]. Addi-

tionally, it influences the activity of immune cells, promoting the differentiation of regulatory T cells (Tregs) while suppressing effector T cell functions, thus maintaining immune tolerance and preventing autoimmunity [30].

TGF-β is also involved in the tissue repair process following injury, where it stimulates the production of ECM components, enhancing angiogenesis and fibroblast proliferation [31]. Its immunomodulatory properties help orchestrate the healing response, thereby ensuring that inflammation resolves appropriately [30]. However, dysregulation of TGF-β signaling can lead to pathological conditions, including fibrosis and cancer.

The role of TGF-β becomes more complex in the context of disease, particularly in cancer, where it can exhibit both tumor-suppressive and tumor-promoting effects depending on the stage of tumor progression and the microenvironment. Initially, TGF-β may function as a tumor suppressor by inhibiting cell proliferation and inducing apoptosis in transformed cells [28]. However, as tumors develop, many cancer cells acquire mutations that enable them to evade TGF-β's growth-inhibitory effects, thereby utilizing TGF-β to promote their survival and metastasis [32,33]. In metastatic disease, particularly within bone metastases, TGF-β plays a key role in establishing a favorable microenvironment for tumor growth [34,35]. Once cancer cells invade the bone, osteoclast-mediated bone resorption leads to the release of latent TGF-β stored in the bone matrix. The reactivated TGF-β then stimulates tumor cells to secrete additional factors that further promote bone degradation and facilitate cancer progression through a "vicious cycle" inhibition of TGF-β results in reduction in bone metastases in several cancer models [36].

TGF-β is particularly implicated in the epithelial-mesenchymal transition (EMT), which enhances cancer cells' invasive and migratory capabilities. This transition is essential for metastasis, enabling cancer cells to detach from the primary tumor and invade surrounding tissues [30,35]. In prostate cancer, for instance, studies have demonstrated that TGF-β signaling enhances the invasive properties of cancer cells, leading to bone metastases and chemoresistance [33,37]. TGF-β–driven EMT is a universal, pan-cancer strategy that tumor cells exploit long before they encounter the bone niche. Large-scale transcriptomic and functional studies in breast, lung, colorectal, and pancreatic carcinomas demonstrate that transient exposure to TGF-β activates core EMT transcription factors (SNAIL, SLUG, ZEB1/2, TWIST), dismantles epithelial junctions, and rewires cytoskeletal and metabolic circuits—changes that endow cells with motility, anoikis resistance, and immune evasion needed for systemic spread. Blocking TGF-β signaling (genetically or with small-molecule/antibody inhibitors) consistently suppresses EMT and sharply reduces metastatic seeding in orthotopic models, regardless of the ultimate destination organ. Thus, while downstream consequences such as osteolysis are bone-specific, the EMT "license to depart" conferred by TGF-β is shared across diverse tumor types and metastatic sites, and should be framed as a general hallmark of the early metastatic cascade rather than a bone-restricted phenomenon [38–40].

2.3 Clinical Significance of TGF-β in Cancer Therapy

Given its dual nature as both a suppressor and promoter of tumorigenesis, targeting the TGF-β signaling pathway has emerged as a potential therapeutic strategy. Rather than completely inhibiting TGF-β, which might disrupt normal healing and immune processes, approaches such as selectively blocking its signaling in the tumor environment may prove beneficial. For instance, Liu *et al.* [41] found that inhibiting TGF-β signaling reduced the incidence of bone metastases in preclinical models. In breast cancer, TGF-β inhibition showed promise in reducing skeletal-related events by interfering with the factors driving bone resorption and oncogenesis [42]. Various studies have shown that combining TGF-β inhibitors with established cancer therapies could enhance antitumor responses with reduced bone-related complications [43]. Additionally, TGF-β's role as a biomarker presents opportunities for improved diagnostic and prognostic assessments in cancer patients. For example, the expression levels of TGF-β and its associated signaling molecules may correlate with disease progression and patient outcomes [44].

3. Bone Metastasis: Pathophysiology and Clinical Impact

Bone metastases are a common phenomenon in advanced cancers, particularly in breast, prostate, and lung cancer. They occur when cancer cells spread to the bone marrow and begin to proliferate, leading to significant complications, including pain, fractures, and systemic symptoms. Understanding the cellular and non-cellular components of the bone metastatic environment is critical for devising therapeutic strategies.

3.1 Mechanisms of Metastatic Spread to Bone

Bone metastasis represents a significant complication in advanced cancer, manifesting a complex interplay of molecular and cellular mechanisms. This process involves interactions between tumor cells and the bone microenvironment, creating a conducive environment for cancer cell survival, colonization, and growth. Specific signaling pathways, cell behavior alterations, and the skeletal microenvironment's dynamic milieu drive the metastasis of various cancers to the bone.

The mechanisms underlying metastatic spread to the bone can be conceptualized as a cascade involving multiple steps, including local invasion, intravasation into the bloodstream, survival in circulation, extravasation into bone, and, ultimately, establishment in the bone microenvironment. In various cancers, including prostate, breast, and lung cancers, the bone microenvironment is enriched with factors

that promote the metastasis process, making it a unique niche for tumor cell colonization [45,46].

Critical pathways implicated in bone metastasis TGF-β signaling, which orchestrates interactions between tumor cells and bone cells, a phenomenon critical for their metastatic success (Fig. 2). Elevated levels of TGF-β have been associated with osteolytic lesions, characterized by excessive bone resorption, an action facilitated by osteoclast activation [47,48]. In prostate cancer, TGF-β signaling enhances the migration and invasion of cancer cells, reinforcing their metastatic potential [49]. In breast cancer, TGF-β signaling acts as a bridge for establishing the bone-pre-metastatic niche, promoting bone colonization by disseminated tumor cells [50,51]. Another essential aspect of bone metastasis is the involvement of microRNAs (miRNAs), which regulate gene expression and consequently influence cancer progression and metastatic behavior. MiR-210-3p has been identified as enhancing the EMT and osteolytic metastasis of prostate cancer via the NF-κB signaling pathway [52]. Similarly, reduced levels of other miRNAs, like miR-133a-3p, have been linked to increased invasiveness and metastatic capability, indicating that miRNAs are crucial players in modulating the molecular landscape supporting metastatic spread [49]. The phenotypic characteristics of metastatic cancer cells provide insights into how tumors adapt to bone. The phenomenon of "osteomimicry", where cancer cells express bone-like characteristics, complicates the metastatic landscape by allowing cancer cells to thrive in the bone microenvironment [46,51]. Cancer cells adopt features such as the production of osteopontin and other bone matrix proteins, which aid in cell adhesion to the bone and promote their survival amidst the challenging conditions prevalent in bone tissue [51]. The process of angiogenesis—the formation of new blood vessels—is significantly enhanced during bone metastasis. Tumor cells influence the local vasculature, encouraging angiogenesis to supply the necessary nutrients for tumor growth and survivability. This interplay between tumor cells and endothelial cells contributes to the complexity of the metastatic drive toward the bone [53]. The immune system plays a paradoxical role in bone metastasis. While some immune components promote tumor clearance, others, particularly those associated with chronic inflammation, may inadvertently facilitate tumor growth and metastasis. Tumor-associated macrophages (TAMs) in the bone marrow can create a favorable niche that enhances the survival and proliferation of metastatic cells, complicating treatment outcomes [53]. Cytokines produced during inflammatory responses, such as interleukin-6 (IL-6), can disrupt normal bone remodeling, promoting osteolytic lesions and enhancing cancer progression [54,55].

The pathobiology of bone metastases is further complicated by the "vicious cycle" of bone metastasis model, which delineates how metastatic cells can induce bone destruction while simultaneously enhancing their own growth [56]. This cycle begins with osteolysis, where cancer cells release factors that stimulate osteoclasts to resorb bone, resulting in the release of growth factors that bolster tumor growth [57]. In prostate cancer, the presence of tumor cells in bone stimulates osteoclast activity leading to increased osteolytic lesions, contributing to severe complications such as SREs [58]. Factors contributing to the bone metastatic process include the expression of specific receptors and adhesion molecules by both cancer cells and bone cells, which govern the dynamics of their interactions. Increased expression of chemokine receptors such as CXCR4 in breast cancer has been linked to enhanced metastatic spread to the bone, suggesting that chemokine signaling is integral to the homing mechanisms of tumor cells [59,60]. Studying bone metastasis also involves various molecular and cellular technologies to unravel the intricate pathways. High-throughput sequencing has identified dysregulated pathways that govern metastatic behavior, which are being explored to develop novel therapeutics targeting these pathways [61,62]. In addition, engineered models simulating the bone microenvironment have contributed to our understanding of how cancer cells adapt and thrive, highlighting potential therapeutic interventions to interrupt these processes [63,64].

Metastatic spread to the bone is a formidable challenge in clinical oncology, characterized by multifaceted mechanisms involving signaling pathways, microenvironmental interactions, immune modulation, and cellular adaptations. The TGF-β signaling pathway, microRNAs, osteomimicry phenomena, and the vicious cycle of bone metastasis are pivotal in understanding and potentially targeting these complex interactions to improve therapeutic outcomes for patients suffering from metastatic bone disease. Ongoing research continues to elucidate these pathways to develop better diagnostic and therapeutic strategies against cancer metastasis to bone.

3.2 Clinical Consequences of Bone Metastases

Bone metastasis presents significant clinical consequences, including pain, fractures, hypercalcemia, and a notably decreased quality of life for affected patients. These sequelae are compounded by the limitations of current therapeutic approaches, highlighting the complexity of managing metastatic bone disease.

One of the most debilitating aspects of bone metastasis is pain. It affects approximately 68–70% of patients, with the severity ranging from mild to intractable [65]. Bone remodeling becomes disrupted when cancer cells invade the bone marrow, leading to the release of inflammatory mediators and the activation of osteoclasts, which exacerbates pain [66,67]. Chronic pain significantly detracts from the quality of life, contributing to physical disability and psychological distress [68,69]. The algorithms defining cancer pain underscore its multifaceted nature, incorporating nociceptive and neuropathic mechanisms [70].

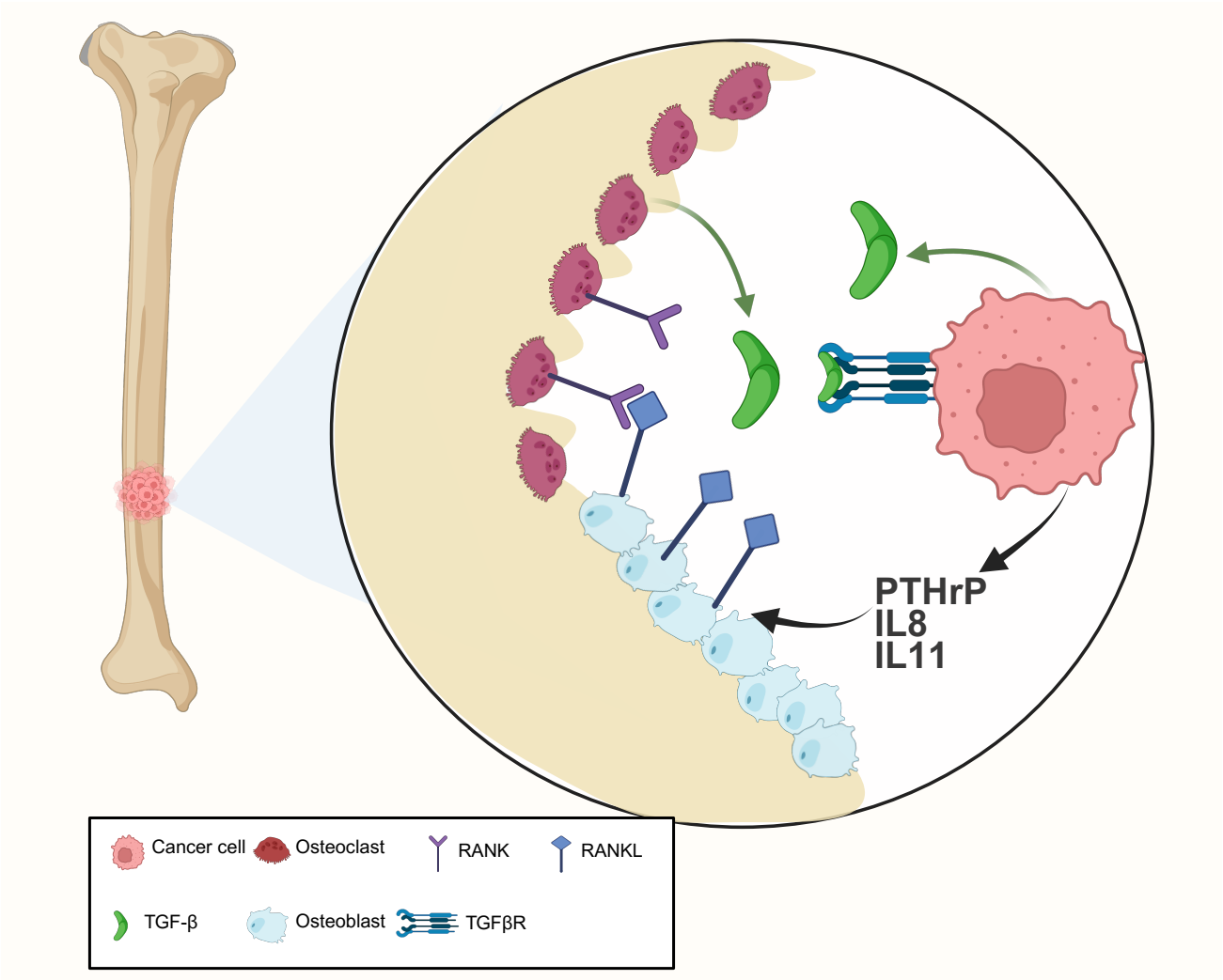

Fig. 2. The TGF-β-centered "vicious cycle" that drives osteolytic bone metastasis. Metastatic cancer cells lodged in bone secrete osteolytic cytokines and growth factors, including parathyroid-hormone-related peptide (PTHrP), interleukin-8 (IL-8) and interleukin-11 (IL-11), that act on neighboring osteoblasts to up-regulate membrane-bound RANK ligand (RANKL). RANKL engages its receptor, RANK, on osteoclast precursors, thereby accelerating osteoclastogenesis and bone resorption. Osteoclastic degradation of the mineralized matrix liberates latent transforming growth factor-β (TGF-β), which is activated in the acidic resorption lacuna and signals through TGF-β receptors (TGFBR) on tumor cells. TGF-β stimulation further amplifies tumor-derived PTHrP, IL-8, and IL-11 expression, reinforcing the osteoblast–osteoclast crosstalk and perpetuating skeletal destruction. The cyclical feed-forward loop simultaneously fuels tumor growth, promotes bone degradation, and releases additional tumor-promoting factors from the matrix, thereby sustaining metastatic colonization. Solid arrows denote stimulatory interactions; curved arrows indicate feedback amplification; dashed lines highlight factors released from resorbed bone. Created in BioRender. Mohammad, K. (2025) https://BioRender.com/xh5psag.

Fractures, especially pathological fractures, represent another major clinical consequence of bone metastasis. Studies indicate that these fractures commonly occur in weight-bearing bones due to the osteolytic activity facilitated by tumor cells [71,72]. Such fractures lead not only to acute pain but also to increased morbidity by requiring surgical interventions or long-term rehabilitation [66,69]. In this context, vertebral fractures can lead to spinal cord compression, creating emergency scenarios that necessitate urgent interventions, thereby affecting mobility and independence [69,73,74]. Hypercalcemia is frequently associated with bone metastasis and occurs when osteoclastic activity leads to excessive calcium release into the bloodstream [73]. Symptoms can be severe, including nausea, vomiting, and confusion, and can even lead to renal failure or cardiac complications if untreated [69]. This condition presents a complex challenge as it often requires immediate medical attention in addition to standard cancer treatment protocols. The impact of bone metastasis extends to a patient's overall quality of life. Research reveals that pain resulting from bone lesions significantly correlates with patients' physical and emotional well-being [66]. The psychological burden

includes anxiety and depression due to chronic pain and disability, further complicating treatment outcomes [70,75]. The multidimensional challenges bone metastases present underscore the necessity for comprehensive management strategies.

3.3 Current Therapeutic Approaches and Limitations

Currently, several therapeutic approaches exist for managing complications arising from bone metastases, primarily focused on palliative care and pain management. These include pharmacological interventions such as bisphosphonates and denosumab, targeted at bone density conservation and pain relief [68,69]. These agents are crucial in moderating SREs and have been shown to significantly reduce the frequency of skeletal complications related to bone metastases [76].

Radiotherapy, particularly palliative radiotherapy, serves a dual purpose: providing pain relief and treating localized tumor burden [74,77]. Single-fraction radiotherapy has emerged as a favorable approach due to its efficacy in relieving pain with minimal intervention [74,78]. However, variability in patient responses necessitates an individualized approach to treatment. Although palliative radiotherapy can lead to improved pain management, most studies reveal that a significant proportion of patients may still experience pain flare-ups post-treatment [79,80].

Despite the advancements in pharmacologic and radiotherapeutic strategies, limitations persist in managing bone metastases. For instance, bisphosphonates do not prevent metastatic spread, nor do they have significant tumoricidal effects [81,82]. While effective for pain management and quality of life improvements, these therapies can sometimes cause adverse events, including renal impairment and osteonecrosis of the jaw [76]. Prior treatments may also limit Radiotherapy, and the presence of widespread metastatic lesions may impact its efficacy [83,84].

Alternative innovations, including image-guided therapies and radiopharmaceuticals like strontium-89 and radium-223, promise to alleviate bone pain associated with metastases; however they also have limitations in terms of side effects and response variability [82,85]. Recently explored modalities, such as high-intensity focused ultrasound and stereotactic body radiotherapy, offer novel approaches for treatment but require further clinical validation before becoming standard practice [86–88]. The empirical approach toward managing bone metastases often highlights the need for an interdisciplinary team, including oncologists, radiologists, pain management specialists, and palliative care experts. This collaborative strategy is essential as it provides comprehensive patient assessment and a tailored approach to managing complex symptoms associated with metastatic bone disease [66,76].

While the clinical consequences of bone metastases include severe pain, fractures, hypercalcemia, and decreased quality of life, current treatments offer significant opportunities for intervention but also face notable limitations. Improving understanding of the underlying mechanisms of pain and developing more effective treatments will be crucial in enhancing outcomes and quality of life for patients with metastatic bone disease.

4. The Tumor Microenvironment in Bone Metastases

The Tumor Microenvironment (TME) in bone metastases is a complex ecosystem that plays a crucial role in determining the success of metastatic colonization and subsequent tumor behaviors. It includes various components, such as tumor cells, stromal cells, immune cells, and the extracellular matrix, all of which interact actively to influence tumor growth, response to treatment, and overall patient outcomes.

4.1 Definition of TME in Bone Metastases

The TME refers to the cellular environment surrounding a tumor, consisting not only of the tumor cells themselves but also of various stromal cells, immune cells, blood vessels, and the ECM [35,89]. In the context of bone metastases, the TME is specifically specialized to support the survival and proliferation of metastatic cancer cells that have colonized the bone tissues [35,90]. The unique architecture and cellular composition of the bone microenvironment create a “niche” that is both supportive and conducive for tumor growth, shaped by the interactions between cancer cells and the local stromal components [91,92].

4.2 Components of the Bone TME

4.2.1 Cellular Constituents in Bone Metastases

The cellular microenvironment in bone metastases comprises several key cellular constituents, including bone-resident cells, hematopoietic and immune cells, and cancer cells. The mesenchymal stem cells (MSCs) reside within the bone marrow and contribute to the structure and function of the bone environment. They play a role in supporting hematopoiesis and in the differentiation of osteoblasts and osteoclasts. Osteoblasts are bone-forming cells that produce the ECM and facilitate mineralization. In the context of bone metastasis, osteoblasts can be influenced by tumor cells through the release of factors such as parathyroid hormone related protein (PTHrP) and TGF-β [93]. These factors promote osteoblastic activity, thereby creating a microenvironment that is conducive to tumor cell survival and proliferation. However, this osteoblastic activation often occurs alongside increased osteoclast activity, leading to mixed lesions where both bone formation and destruction occur [94,95]. Osteoclasts are responsible for bone resorption, and their activity is crucial in the pathogenesis of bone metastases [94,95]. Upon cancer invasion, these MSCs can adopt a pro-tumor phenotype, facilitating the growth of metastases through paracrine signaling, which includes the secretion of cytokines such as IL-6 and TGF-β,

which are often implicated in promoting tumor progression [96,97]. The bone marrow is rich in hematopoietic cells, including macrophages, T cells, and other immune cell types. These immune cells can create a pro-inflammatory microenvironment that supports tumor growth and protects cancer cells from immune-mediated destruction. For instance, bone-resident macrophages can exhibit a unique polarization that promotes the local metastatic spread of cancers, including breast and prostate cancer [98,99]. Myeloid-derived suppressor cells (MDSCs) are often recruited to the metastatic niche and play a significant role in immunosuppression during cancer progression [99]. The cancer cells that metastasize to bone often undergo a transformation that prepares them for survival and growth in the bone microenvironment. This involves not only the reactivation of signaling pathways previously inhibited by TGF-β but also the expression of specific molecules that facilitate interactions with the bone matrix and stromal cells [97,100]. For example, breast cancer cells express molecules such as RANKL and PTHrP, which further enhance osteoclast activity and facilitate a cycle of bone resorption and tumor growth [96,97].

4.2.2 Non-Cellular Components of Bone Metastases

In addition to cellular constituents, the non-cellular components also play a significant role in the bone metastatic microenvironment. These include cytokines, growth factors, and the ECM. Cytokines are signaling molecules that modulate the interactions between cancer cells and the host's bone environment. TGF-β is a potent cytokine released from the bone matrix during bone resorption, and it has diverse roles in enhancing tumor cell proliferation, invasion, and metastasis [96]. Other important cytokines include IL-6, which contributes to an inflammatory loop boosting cancer progression, and Insulin-like Growth Factor (IGF), which mediates the crosstalk between cancer cells and bone tissue [97]. The ECM provides structural support to tissues and is comprised of various proteins, including collagen, osteopontin, and fibronectin. Each of these matrix proteins significantly influences the behavior of cancer cells in the bone. Collagen is the predominant protein in the ECM, collagen serves as a scaffold for cell attachment and influences cellular behavior through integrin signaling pathways. Cancer cells can exploit the ECM to drive their invasiveness, as modified collagen structures can promote cancer cell migration and resistance to apoptotic signals [96,101]. Osteopontin is a non-collagenous glycoprotein that is present in the bone and plays a critical role in cell adhesion and signaling. High levels of osteopontin are associated with increased metastatic potential of cancer cells. It aids in cell proliferation and survival and is often upregulated in metastatic bone lesions [102,103]. Fibronectin is another essential ECM protein, fibronectin is involved in cell attachment and migration. In conjunction with other growth factors, fibronectin contributes to the formation of a pro-tumor environment by enhancing the anchoring of cancer cells to the bone matrix, modulating their signaling pathways, and promoting a mesenchymal phenotype critical for metastasis [104,105].

The matrix proteins provide structural support and actively modulate the behavior of cancer cells. Cancer cells utilize integrins to anchor themselves to fibronectin or osteopontin in the ECM. This adhesion is crucial for their subsequent migration and invasion into surrounding tissues [96,101]. The presence of specific matrix proteins, such as TGF-β and some collagen types, can induce epithelial-mesenchymal transition (EMT) in cancer cells, thereby enhancing their metastatic capabilities and resistance to therapies [97,106]. The ECM environment can provide survival signals through various pathways, including those mediated by integrins. Cancer cells that establish strong interactions with the ECM are often more resistant to apoptotic signals and can lead to treatment resistance [104,105].

The primary components of the TME are the metastatic cancer cells themselves, which may exhibit altered phenotypes compared to their origins, often adopting characteristics that favor invasion and survival in the bone environment [107,108]. For instance, breast cancer cells can undergo an EMT to increase their invasive potential and adapt to the bone stroma [108,109]. The bone TME comprises various stromal cells, including osteoblasts, osteoclasts, and fibroblasts. Osteoblasts are involved in bone formation, while osteoclasts are responsible for bone resorption [110]. Tumor cells can manipulate these cells to create a favorable environment for metastasis; they may stimulate osteoclasts to enhance bone resorption, leading to osteolytic lesions typically seen in metastatic disease [111,112]. This interaction results in a vicious cycle characterized by tumor-induced bone remodeling and further tumor growth [113]. The TME in metastatic bone can contain various immune cell types, including TAMs, T cells, and other lymphoid cells. Macrophages within the TME can actively promote tumor growth by releasing pro-inflammatory cytokines, which can enhance tumor cell proliferation and survival [114–116]. They also create an immunosuppressive environment that allows for tumor evasion from immune surveillance [117,118]. TAMs, specifically M2-type macrophages, are often enriched in bone metastases and are associated with promoting tumor growth and progression via their secreted factors [118,119].

The ECM within the bone TME provides structural support and biochemical signals crucial for maintaining tumor cell survival and proliferation [107,120]. Changes in the ECM composition, driven by factors released from both tumor cells and stromal components, can facilitate the invasion of cancer cells and modify their interactions within the bone [121]. Matrix proteins, such as collagen and fibronectin, can influence the behavior of cancer cells, including their migration and the signaling pathways involved in metastasis.

The bone TME is rich in cytokines and growth factors that play a crucial role in stimulating tumor cell growth and survival. Key players include Transforming Growth Factor beta (TGF-β), which is released during bone resorption and can stimulate metastatic tumor cells to secrete additional factors that promote further bone destruction [35,107,122]. Other factors, such as PTHrP, are also implicated in promoting osteoclast activation and thus play a role in the osteolytic process associated with bone metastasis [123]. Communication between and among the different cell types in the bone TME is critical for establishing and progressing metastasis. Interactions mediated by integrins, cadherins, and growth factor receptors contribute to the plasticity of metastatic cells, allowing them to adapt to the specific microenvironment of the bone [91,124]. Such interactions not only enhance metastatic potential but also can lead to treatment resistance, as the TME can hinder the efficacy of conventional therapies [125,126].

The TME in bone metastases represents a highly dynamic and interactive environment that profoundly affects tumor behavior and treatment responses. Understanding its complex components and their interactions is essential for developing therapeutic strategies aimed at inhibiting bone metastasis and improving patient

4.3 Role of the Bone Microenvironment in Supporting Tumor Colonization and Growth

The bone microenvironment plays a pivotal role in supporting tumor colonization and growth, particularly in the context of metastatic disease. The unique cellular composition, including osteoblasts, osteoclasts, osteocytes, immune cells, and various stromal components, creates a supportive niche for tumor cells. The interactions within this microenvironment facilitate tumor growth, modify local bone remodeling processes, and promote the establishment of secondary metastatic lesions.

Tumor cells can stimulate osteoclastogenesis through RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) expression, which binds to RANK receptors on osteoclast precursors, promoting their differentiation and activation [127]. This resorption leads to the release of growth factors stored within the bone matrix, such as TGF-β and Bone Morphogenetic Proteins (BMPs), further enhancing the growth of metastatic tumor cells and perpetuating a vicious cycle of bone degradation and tumor advancement [48,128] (Fig. 3). Thus, the interplay between osteoblasts and osteoclasts facilitates metastasis and drives the bone's ongoing remodeling, highlighting their essential role in the tumor microenvironment (TME). Osteocytes, the most abundant cells in mature bone, are embedded in the mineralized matrix and play critical roles in mechanosensation and signaling [129]. While their role in direct tumor interactions is less well characterized, osteocytes can influence the behavior of osteoclasts and osteoblasts through signaling pathways such as sclerostin and fibroblast growth factor (FGF) signaling [128]. By regulating local bone remodeling, osteocytes can indirectly support tumor colonization.

The immune landscape in the bone microenvironment is also crucial for tumor colonization and growth. Various immune cells, including TAMs, play a dual role in bone metastases, either promoting or inhibiting tumor growth. TAMs are often polarized towards a pro-tumorigenic M2 phenotype in the presence of cancer cells, which leads to increased inflammation, matrix remodeling, and further recruitment of additional immune cells [110,130]. This polarization can be enhanced through bidirectional signaling involving cytokines and chemokines produced by both the tumor and stromal cells [130,131]. Stromal cells, including cancer-associated fibroblasts (CAFs) and mesenchymal stem cells (MSCs), also significantly influence tumor dynamics in the bone microenvironment. MSCs can differentiate into various cell types, including osteoblasts, and release paracrine factors that support cancer cell survival, proliferation, and migration [132,133]. CAFs contribute to the fibrotic response and create a supportive extracellular matrix, critically altering cell interactions and promoting tumor growth [133,134]. The interaction between tumor cells and the stromal components can activate various signaling pathways that facilitate proliferation and survival. For instance, the interaction of Jagged1 and Notch signaling in bone cells has been shown to enhance osteolytic metastasis by promoting osteoclast differentiation and activity [135,136]. Furthermore, factors such as IL-6 are secreted by both tumor and stromal cells, contributing to a pro-inflammatory environment that favors metastasis [48,110,137].

The bone microenvironment is a highly orchestrated assembly of specialized cells that provide a supportive niche for tumor colonization and growth. Osteoblasts, osteoclasts, and osteocytes interact dynamically with immune components and stromal cells to create an ecosystem that promotes cancer cell survival and proliferation. These interactions generate a vicious cycle of bone remodeling and metastasis that complicates treatment strategies aimed at managing bone-associated cancers. Thus, understanding the complexities of the bone microenvironment is critical for developing novel therapeutic approaches that target these interactions to hinder tumor growth and improve patient outcomes.

4.4 Signaling Pathways Involved in TME Modulation

The signaling pathways involved in modulating the TME in bone metastases play critical roles in facilitating the growth and survival of metastatic cancer cells. Several pathways interact with bone-specific cell types and immune components, contributing to the complexities of cancer progression and treatment resistance. Below, we discuss prominent signaling pathways that are implicated in TME modulation specifically related to bone metastases.

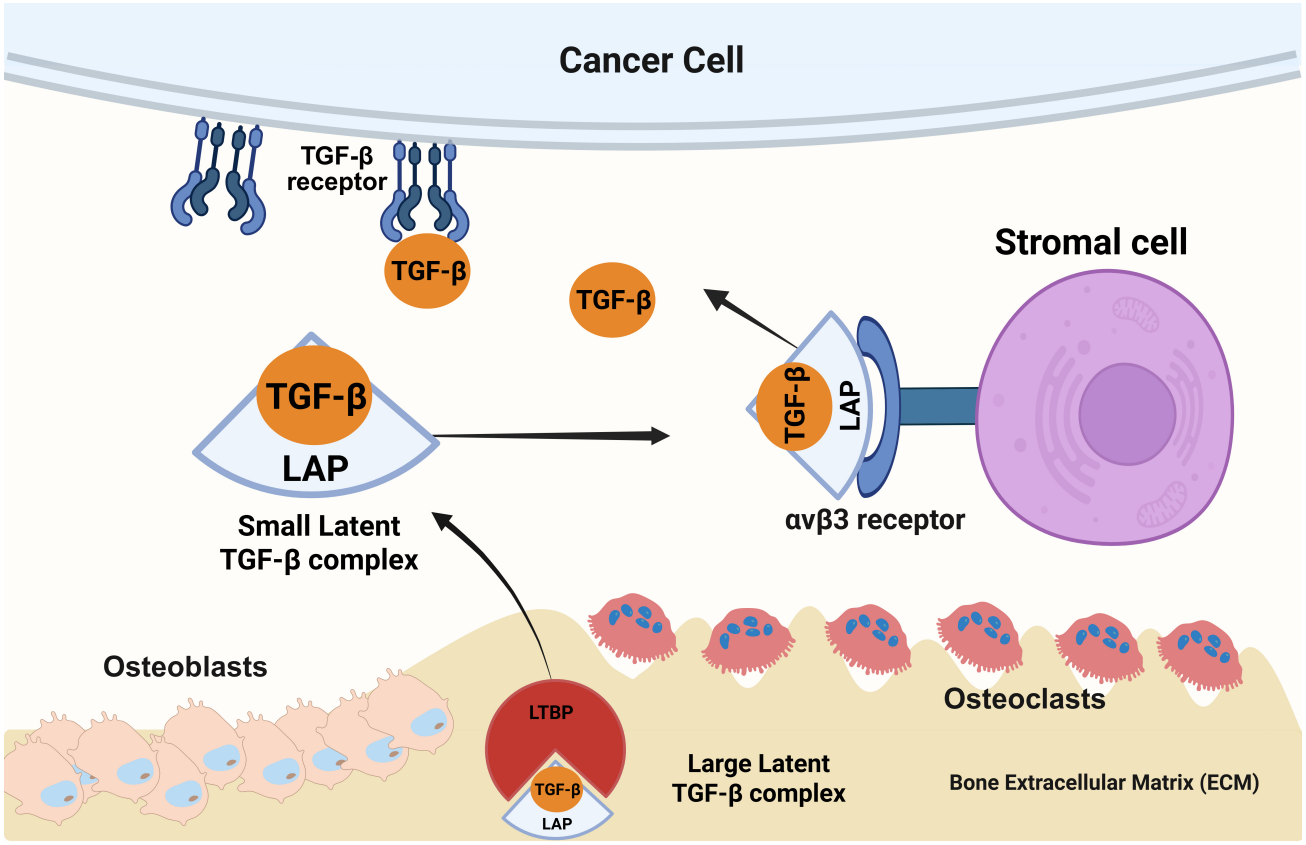

Fig. 3. How inactive TGF-β is activated in bone and transformed into a pro-metastatic signal. In healthy bone, most TGF-β is stored in the extracellular matrix (ECM) as a large latent complex in which the cytokine (blue) is enveloped by its latency-associated peptide (LAP, grey) and tethered to the matrix via latent TGF-β-binding protein (LTBP, orange). When osteoclasts resorb bone or when mechanical stress or proteases remodel the matrix, the complex is partially cleaved, yielding a small latent complex (with TGF-β still encased by LAP). Tumor cells, osteoclasts, and osteoblasts that express the αvβ3 (and related αvβ6/αvβ8) integrins bind LAP, and by exerting traction, they mechanically unfold it, releasing mature TGF-β. The liberated ligand engages TGF-β receptors on cancer cells and stromal cells, triggering SMAD-dependent transcriptional programs that foster tumor growth, osteoclastogenesis, and immune suppression. Thus, what begins as an inert growth factor buried in the bone matrix becomes a powerful driver of the metastatic cascade through sequential proteolysis and integrin-mediated activation. Created in BioRender. Mohammad, K. (2025) https://BioRender.com/z6unrvc.

4.4.1 Transforming Growth Factor-beta (TGF-β) Signaling Pathway

TGF-β is a multifunctional cytokine that plays a central role in the TME in various cancers, including bone metastases. It is secreted by both tumor cells and stromal cells and influences numerous cellular processes, including proliferation, differentiation, and apoptosis [138, 139]. TGF-β signaling is known to induce EMT, enhancing cell invasiveness and promoting metastatic capabilities [140,141]. In bone metastasis, TGF-β can induce a local environment conducive to tumor cell survival and further dysfunction of bone remodeling [35,141].

4.4.2 Bone Morphogenetic Protein (BMP) Signaling Pathway

BMPs are crucial regulators of bone homeostasis and have been implicated in cancer-induced bone metastasis through their roles in osteogenesis and in promoting tumor cell interactions with bone cells [140,142]. BMP signaling functions are closely linked to TGF-β signaling and can have both pro-tumorigenic and anti-tumorigenic effects, depending on the context [42]. In breast cancer, the aberrant activation of BMPs has been shown to promote osteolytic lesions, resulting in a supportive TME for metastatic growth [142]. In prostate cancer, BMP signaling drives osteoblastic changes that can alter the TME, enhancing tumor aggressiveness [142].

4.4.3 Wingless/Integration-1 (Wnt)/β-Catenin Signaling Pathway

The Wnt signaling pathway plays a critical role in maintaining bone homeostasis and influencing tumor biology. In the context of bone metastases, Wnt signaling induces the differentiation of osteoblasts, enhancing bone formation and providing a favorable environment for tumor cells [143–145]. Aberrant Wnt/β-catenin signaling

has been shown to result in M2 macrophage polarization, fostering immunosuppression in the bone microenvironment. Targeting this pathway using inhibitors has been explored as a therapeutic strategy in treating bone metastases, highlighting its significant role in tumor-stroma interactions [143,144].

4.4.4 NF-κB Signaling Pathway

Nuclear factor-kappa B (NF-κB) is a transcription factor that is activated in many cancers, regulating genes associated with inflammation, immunity, and cell survival. In the TME, NF-κB activation can promote the recruitment of immune cells and support tumor growth by fostering a supportive microenvironment for metastasis [146,147]. In osteolytic metastases, NF-κB contributes to osteoclast activation, thereby participating in the vicious cycle of bone destruction and tumor progression [146,148]. This pathway is also involved in regulating the responses of tumor-associated macrophages (TAMs), further perpetuating an immunosuppressive microenvironment conducive to metastatic spread [146].

4.4.5 PI3K/Akt Signaling Pathway

The PI3K/Akt signaling pathway is a major regulator of cell proliferation and survival, and it becomes dysregulated in various forms of cancer, including those with bone metastases [149]. In prostate cancer specifically, the pathway has been implicated in the promotion of osteogenesis from mesenchymal stem cells (MSCs), further modifying the TME to favor cancer progression [149,150]. Akt signaling is vital for osteoclast differentiation and activation, thus contributing to bone resorption and remodeling associated with metastatic lesions [149].

4.4.6 JAK/STAT Signaling Pathway

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is often activated in various cancers and is crucial for mediating responses to inflammatory cytokines in the TME. In the case of bone metastases, such signaling facilitates the transition of macrophages to an M2 phenotype, enhancing the immunosuppressive environment that tumor cells can exploit for growth and survival [122]. The activation of this pathway through cytokine signaling allows cancer cells to evade immune surveillance mechanisms, which is particularly detrimental in the context of bone metastasis [122,151].

4.4.7 Chemokine Signaling Pathway

The chemokine signaling pathway, particularly via the CCR2 receptor and its ligand CCL2, plays an important role in the recruitment of immune and stromal cells to the TME. These cells can enhance tumor growth and survival through various mechanisms, including the engulfment of tumor cells and the modulation of inflammation [152]. In the context of bone metastases, chemokine-driven cell recruitment can alter the microenvironment's composition, creating a more favorable niche for tumor growth.

The tumor microenvironment in bone metastases is modulated by diverse signaling pathways, including TGF-β, BMP, Wnt/β-catenin, NF-κB, PI3K/Akt, JAK/STAT, and chemokines. These pathways not only affect the behavior of metastatic tumor cells but also influence the surrounding bone and immune cells, creating a dynamic ecosystem that supports tumor colonization and growth. Understanding these pathways provides critical insights for developing targeted therapies that can disrupt these interactions and improve treatment outcomes for patients with bone metastases.

5. Central Role of TGF-β in the Bone Metastatic Niche

The sources of activation for TGF-β in bone metastases primarily include the bone matrix and osteoclastic activity, which release latent TGF-β upon degradation of the extracellular matrix during bone resorption. Initially, TGF-β is secreted in an inactive form and is stored within the mineralized bone matrix. When osteoclasts engage in bone resorption, they release active TGF-β into the microenvironment of the tumor, where it can exert its metastatic effects on cancer cells (Fig. 2). For instance, osteolysis-induced TGF-β activity stimulates cancer cell proliferation and induces further osteoclastogenesis through the upregulation of RANKL by osteoblasts [44,153]. This interplay demonstrates a feedback loop where TGF-β enhances both the resorptive activity of osteoclasts and the aggressiveness of the metastasizing tumor cells.

TGF-β signaling is mediated through the SMAD family of proteins, particularly SMAD2 and SMAD3, which are phosphorylated following TGF-β receptor activation [42]. These phosphorylated proteins translocate to the nucleus where they function as transcription factors to induce the expression of various target genes that facilitate tumor metastasis and EMT [37,41]. The role of TGF-β in facilitating EMT is particularly significant, as it promotes the invasive properties of tumor cells, aiding their survival and proliferation in the hostile bone microenvironment. In addition to the osteoclastic source of TGF-β, other cellular mechanisms contribute to its activation in bone metastases. For example, genetic and epigenetic factors influencing TGF-β signaling can modulate its activity in tumor cells. Alterations in key signaling proteins, such as 14-3-3ζ, can shift TGF-β's role from a tumor suppressor to a promoter of metastasis, while microRNAs like miR-19a-3p can also regulate TGF-β signaling pathways, enhancing the migratory capabilities of cancer cells [32,154]. Integrins, particularly αv integrin, have been implicated in TGF-β activation, serving as critical mediators linking extracellular matrix interactions with intracellular TGF-β signaling [155].

5.1 Biology and Signaling Mechanisms of TGF-β

In the initial stages of tumor development, TGF-β can function as a tumor suppressor by inhibiting excessive cell proliferation and inducing apoptosis in premalignant cells [156,157]. However, as tumors progress to metastatic stages, TGF-β often switches its role, promoting invasion and metastasis. This shift is facilitated by TGF-β's involvement in EMT, a critical process that enables cancer cells to acquire migratory and invasive properties [41,158]. EMT is predominantly driven by the activation of SMAD proteins, particularly SMAD2 and SMAD3, which translocate to the nucleus upon TGF-β receptor activation, leading to the expression of pro-migratory and pro-metastatic genes [32,41]. The activation of TGF-β in the bone microenvironment is predominantly influenced by osteoclastic activity, which releases latent TGF-β from the mineralized bone matrix during the bone resorption process. This, in turn, enhances metastatic cancer cell proliferation and further stimulates osteoclastogenesis through PTHrP secretion by tumor cells [96,154]. This "vicious cycle" between bone resorption and tumor cell proliferation significantly contributes to bone metastasis [96,159]. The regulatory landscape of TGF-β signaling is complex, featuring several feedback mechanisms that can enhance or inhibit its activity. For example, the negative regulators of TGF-β pathways, such as PMEPA1 and PICK1, can attenuate TGF-β-mediated processes when activated [32,37]. Disruption of these regulatory feedback loops can lead to enhanced TGF-β signaling and increased metastatic potential, underscoring the dual nature of TGF-β in tumor biology [160,161]. Moreover, recent studies have highlighted the role of microRNAs in modulating TGF-β signaling. MicroRNAs such as miR-19a-3p have been shown to promote invasion and migration through TGF-β pathway activation in prostate cancer [32]. Additional mechanisms through which TGF-β promotes bone metastasis include the stimulation of matrix metalloproteinases (MMPs) that degrade the extracellular matrix, facilitating tumor invasion into bone [162,163]. Furthermore, the interaction of with fibroblast growth factors (FGFs) in myeloid cells enhances the metastatic process, indicating that myeloid-specific TGF-β signaling plays a crucial role in bone metastasis [44,159].

5.2 Effects of TGF-β on Tumor Cells and the Bone Microenvironment

TGF-β influences spans various biological processes, affecting both tumor cells and the bone microenvironment. This includes effects on tumor cell dormancy and reactivation, induction of EMT, regulation of osteoclastogenesis and osteoblast suppression, and immunosuppressive effects within the tumor microenvironment (TME). TGF-β is known to induce EMT, facilitating the transition of epithelial cells into a mesenchymal phenotype, which enhances tumor cell invasion and metastasis. This effect is particularly salient in breast cancer, as TGF-β can drive bone metastasis through mechanisms involving the induction of osteolytic factors such as interleukin-11 (IL-11) that demineralize bone and promote tumor invasiveness [34,164]. Furthermore, TGF-β signaling is intricately connected to the survival of tumor cells in a dormant state, as it is released from the bone matrix upon osteoclastic resorption, creating a niche that can reactivate dormant cancer cells as seen in bone metastasis [34,107]. The cyclic interaction between TGF-β release and tumor cell reactivation establishes a problematic vicious cycle, perpetuating tumor growth and metastasis in the bone environment [34,107].

Regarding osteoclastogenesis, TGF-β initiates signaling pathways that enhance the differentiation and activity of osteoclasts, the cells responsible for bone resorption. TGF-β acts synergistically with receptor activator of nuclear factor-kappa B ligand (RANKL) to stimulate osteoclast formation and function, promoting osteolytic lesions commonly seen in metastatic cancer [165,166]. Studies indicate that TGF-β enhances the expression of key genes associated with osteoclast activity, such as NFATc1 and TRAP, thereby facilitating increased osteoclastogenesis [167,168]. In contrast, TGF-β's role in osteoblasts (the bone-forming cells) is inhibitory; it suppresses osteoblast differentiation and mineralization, which hinders new bone formation [29,169].

The immunosuppressive effects of TGF-β within the TME are critical. TGF-β can modulate the immune response, promoting a tumor-friendly environment by inhibiting the activation and proliferation of various immune cells. It has been shown to induce regulatory T cells while suppressing the functions of cytotoxic T cells and natural killer cells [34,107]. This immunosuppressive landscape created by TGF-β plays a significant role in the tumor's ability to evade immune surveillance, further contributing to the metastatic potential of cancer cells. TGF-β serves as a pivotal mediator within the tumor and bone microenvironments, driving tumor growth and metastasis through its roles in cell transition, osteoclast activation, osteoblast suppression, and immune modulation. Addressing the effects of TGF-β holds substantial potential for therapeutic interventions aimed at mitigating its contributions to cancer progression and bone-related complications.

6. Therapeutic Targeting of TGF-β Signaling in Bone Metastases

6.1 Overview of Current Strategies Targeting TGF-β Signaling

TGF-β is a critical mediator in cancer progression and metastasis, which has led to the development of several strategies aimed at inhibiting its signaling pathway. These strategies include the use of monoclonal antibodies against TGF-β ligands and receptors, small molecule inhibitors of TGF-β receptors, and antisense oligonucleotides (ASOs) and RNA interference (RNAi).

Monoclonal antibodies targeting TGF-β ligands and receptors are designed to block ligand-receptor interactions, thereby inhibiting downstream signaling pathways that promote tumor growth and metastasis. Antibodies such as fresolimumab and lerdelimab have shown promise in preclinical and clinical studies, exhibiting potential in various malignancies by diminishing TGF-β activity in the tumor microenvironment. These antibodies aim to disrupt the autocrine and paracrine signaling loops facilitated by TGF-β, thereby reducing tumor-associated immunosuppression and enhancing antitumor immunity, as evidenced in studies addressing pancreatic and breast cancers [170–173].

Small-molecule inhibitors, such as ALK5 inhibitors, are also critical in targeting TGF-β signaling. These compounds act primarily on TGF-β type I receptors, preventing their activation and subsequent downstream signaling via the Smad pathway. Novel small molecules, such as galunisertib and Vactosertib, have been developed and tested in animal models, showcasing diminished tumor growth and increased efficacy in combination with other therapies [172,174]. Additionally, the potential of combining these inhibitors with immune checkpoint inhibitors has emerged, as they could synergistically enhance therapeutic efficacy in previously resistant tumors [175,176].

Antisense oligonucleotides and RNA interference, such as Trabedersen and ISTH0036, represent another innovative approach to inhibit TGF-β signaling. ASOs are designed to bind specifically to TGF-β mRNA, thereby preventing translation and subsequently reducing TGF-β protein levels in the tumor microenvironment [177]. Recent studies have demonstrated that targeting TGF-β2 with ASOs enhances the T cell-mediated anticancer efficacy of therapies like anti-PD-1 in murine models, suggesting a potential for combining ASOs with existing immunotherapies [171,178]. RNA interference has also been explored for its ability to silence genes involved in TGF-β signaling, presenting an opportunity to downregulate pro-tumorigenic effects and re-sensitize tumors to chemotherapy [179,180].

TGF-β is essential for both wound repair and fracture healing. TGF-β is integral to granulation tissue formation, collagen synthesis, and epithelialization [181]. TGF-β signaling regulates matrix metalloproteinases (MMPs), which are necessary for cell migration and tissue remodeling during repair. TGF-β is predominantly secreted by platelets and osteoblasts during the early phases after injury, promoting the recruitment of mesenchymal stem cells (MSCs), chondrocytes, and other crucial cell types that facilitate healing [182]. Global blockade in case of bone metastases poses significant risks to these biological processes. Tumor-selective ligand traps represent a promising strategy that could mitigate the negative consequences associated with broad TGF-β inhibition, thus allowing for both effective cancer management and the preservation of normal healing mechanisms.

Targeting TGF-β signaling through these diverse strategies offers a promising avenue for cancer therapy. Ongoing clinical trials and preclinical studies continue to elucidate the precise role of TGF-β in cancer biology and the effectiveness of these therapeutic approaches, potentially leading to more effective treatments for patients with advanced malignancies.

6.2 Clinical Studies and Outcomes

The role of TGF-β in the context of bone metastases and cancer is an area of active clinical investigation. TGF-β is known for its dual role in cancer biology, acting as both a tumor suppressor in early stages and promoting metastasis and immune suppression in advanced disease stages [35,183]. This duality presents challenges in developing effective therapeutic strategies that target TGF-β signaling.

Previous studies demonstrated that TGF-β signaling is frequently activated in metastatic bone lesions. In a study by Liu *et al.* [41], a significant percentage of metastatic prostate cancer cells in bone were found to have phosphorylated Smad2, indicating active TGF-β signaling, which is crucial for promoting bone metastasis. Similarly, Zhang *et al.* [37] identified the TGF-β/KLF5 axis as a key mechanism facilitating epithelial-mesenchymal transition (EMT) in bone metastatic prostate cancer, contributing to chemotherapy resistance.

Efforts to target the TGF-β pathway in clinical settings have shown promise. For instance, Khaddour *et al.* [184] discussed worse survival outcomes associated with bone metastases in different cancers, suggesting that TGF-β may contribute to a more aggressive tumor microenvironment that is immunosuppressive and facilitates tumor progression. Verbruggen *et al.* [185] highlighted a potential therapeutic avenue through inhibiting the TGF-β pathway, demonstrating that targeting this pathway could disrupt a feedback loop that enhances metastatic behavior.

The targeting of TGF-β has yielded both successes and limitations. Successful inhibition of TGF-β signaling, such as through the use of neutralizing antibodies or TGFBR1 inhibitors, has shown effectiveness in preclinical models by reducing osteolysis and improving bone density in models of metastatic breast cancer [186]. However, challenges remain due to the complex role of TGF-β. In systems where TGF-β was inhibited, compensatory mechanisms often emerged, with some studies indicating that additional pathways could be activated which mitigate the therapeutic effects [187].

TGF-β's role in immune suppression complicates treatment. It inhibits T-cell activation and promotes regulatory T-cell development, which can hinder the effectiveness of immunotherapies [183]. Therefore, while targeting TGF-β may improve outcomes in some patients, it may concurrently worsen the immune landscape or promote resistance, necessitating careful patient selection and combinatorial treatment approaches.

Several ongoing clinical trials are exploring the targeting of TGF-β in the context of cancer with bone metastases. The biopharmaceutical SYN101, a first-in-class immune cell-targeted TGF-β inhibitor, is being tested for its ability to block immune suppression while enhancing anti-tumor immunity [187]. Additionally, oral inhibitors of TGF-β receptor (such as Vactosertib) are being explored for their potential to promote anti-tumor immune responses in patients with bone malignancies like osteosarcoma, highlighting the evolving nature of clinical strategies aimed at manipulating TGF-β pathways [188].

TGF-β remains a critical target in combating bone metastases, with ongoing studies aimed at deciphering its complex roles and effects. While inhibitors have shown potential in preclinical and early clinical settings, further research is needed to understand how to best navigate the dual pathways of TGF-β's actions in tumor progression and immunity to design effective therapeutic strategies.

7. Beyond TGF-β: Additional Targets in the Tumor Microenvironment

7.1 Osteoclast and Osteoblasts Targeting Therapies

One promising approach to target the bone microenvironment involves osteoclast-targeting therapies, such as RANKL inhibitors, with Denosumab being a notable example. RANKL (Receptor Activator of Nuclear factor Kappa-β Ligand) plays a crucial role in osteoclast differentiation and activation, which are essential processes for bone resorption and the establishment of a microenvironment conducive for tumor growth [189,190]. By inhibiting RANKL, Denosumab serves to reduce osteoclast activity, thereby potentially decreasing osteolysis associated with bone metastases and improving patient outcomes [191]. Studies have shown that the inhibition of RANKL not only reduces skeletal-related events in patients with bone metastases but also demonstrates a protective effect against cancer-induced bone loss [191,192].

In addition to RANKL inhibition, the modulation of osteoblast activity is crucial in bone metastatic contexts. Tumors can educate osteoblasts to adopt traits that benefit tumor growth, such as increased bone formation leading to osteoblastic lesions [193,194]. The identification of receptors and signaling pathways that facilitate this crosstalk presents another avenue for therapeutic intervention. For instance, the role of parathyroid hormone-related peptide (PTHrP) is significant as it upregulates RANKL in osteoblasts, promoting a feed-forward loop that exacerbates osteoclast activity and further stimulates tumorigenesis in the bone [91,193]. Targeting PTHrP could reduce osteoclastogenesis and, consequently, the destructive effects of cancer metastases in bone. Stromal cell modulation offers an innovative therapeutic strategy by altering the supportive network that facilitates metastatic growth. The bone stroma itself can influence cancer cell behavior through the release of chemokines and growth factors, enhancing tumor cell migration and proliferation within this niche [195,196]. For example, the interaction of prostate cancer cells with bone stromal cells has been shown to activate osteoblasts and promote osteoblastic responses, thus changing the bone microenvironment to one that is more conducive to metastasis [101,197]. Interventions that disrupt these stromal-tumor interactions may lead to reduced metastatic progression.

Therapies targeting the immune components within the bone microenvironment could be advantageous. Investigating the immune landscape, including immune checkpoint inhibitors or agents that modulate myeloid-derived suppressor cells and regulatory T cells, reveals potential strategies to enhance anti-tumor immunity while countering the immune-suppressive effects that bone metastases can elicit [198]. This not only holds promise for reducing tumor growth but also for restoring normal bone remodeling processes that are often disrupted by metastatic lesions.

7.2 Immune Checkpoint Inhibitors (ICI) and Combination Therapies

One major challenge with using Immune Checkpoint Inhibitors (ICIs) in bone metastases stems from the immunosuppressive microenvironment inherent to bone, characterized by regulatory T cells and other factors that can inhibit effective immune responses [199,200]. Studies indicate that bone metastases often show diminished immune activity compared to primary tumors, complicating treatment outcomes [201]. Nonetheless, emerging strategies suggest promise for ICIs even in this challenging context.

Recent clinical case reports have documented complete remission in patients with renal cell carcinoma and melanoma who showed strong responses to ICIs despite having advanced bone metastasis. These results emphasize the potential of ICIs to produce meaningful responses, indicating that even patients with bone metastases may benefit from immunotherapies under appropriate clinical circumstances. Moreover, the presence of immune checkpoint expression (such as PD-1 and PD-L1) within bone metastatic lesions suggests that targeting these pathways could enhance response rates [99,201].

Combination therapies involving ICIs are showing promise in enhancing therapeutic effectiveness. For example, combining radiation therapy with ICIs has been explored for its potential to induce an "abscopal effect", where local treatment triggers systemic anti-tumor responses, which could be beneficial for bone metastases [202,203]. This is particularly relevant as radiation can make the tumor microenvironment more immunogenic, thereby enhancing the effectiveness of subsequent ICI therapy [204]. Combining ICIs with traditional systemic agents, such as tyrosine kinase inhibitors or other immunomodulatory treatments, provides another approach to improve outcomes. These strategies can target both the cancer cells and the supporting stroma in the bone microenvironment [205,206]. Notably, dual-combination therapies (e.g., anti-PD-1 and anti-

CTLA-4) have been shown to improve survival outcomes in advanced melanoma, demonstrating a concept that could be applied to treatments involving bone metastases [207]. While immune checkpoint inhibitors present challenges in bone metastases due to the complex immunosuppressive environment, ongoing research and clinical experience indicate that ICIs can offer therapeutic options for certain patients, particularly when combined with other treatments.

T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) has been identified as a checkpoint that plays a significant role in T-cell exhaustion during chronic infections and cancer [208]. Its expression on T cells can inhibit effective anti-tumor responses, which is particularly relevant in the metastatic niche where the immune system may be suppressed. In the context of bone metastases, where the immune microenvironment can be influenced by factors such as osteoclast activity, targeting TIM-3 may present a strategic opportunity to reinvigorate T-cell responses against metastatic tumor cells [199]. Studies have shown that TIM-3 expression can correlate with tumor progression and poor prognosis in various cancers, linking its elevated levels to reduced overall survival in patients with bone metastases [209].

Lymphocyte-activation gene-3 (LAG-3), another critical immune checkpoint, functions similarly as an inhibitory receptor on T cells and is thought to be co-expressed with PD-1 in the tumor microenvironment [210]. LAG-3's importance has been illuminated in contexts such as renal cell carcinoma, where increased expression has been associated with metastatic disease [211]. Research indicates that LAG-3 has a unique expression pattern in metastatic tissues compared to primary tumors, influencing patient outcomes and therapeutic responses [210,212]. The combined expression of LAG-3 with other immune checkpoints like PD-1 has been linked to immune evasion mechanisms, particularly in metastasis, suggesting that simultaneous targeting of these markers may improve immunotherapy efficacy [213]. Recent investigations have also highlighted the significance of the bone microenvironment in modulating immune checkpoint regulator expression. Factors in the bone niche, such as osteoclast and osteoblast signaling, influence the local immune landscape, making the study of specific immune checkpoints like TIM-3 and LAG-3 particularly relevant to metastatic processes [214,215]. Research into the Siglec-15/sialic acid glycoimmune checkpoint axis has further exposed pathways by which bone metastases can alter immune responses via inhibition of T-cell activity, underscoring the necessity of a comprehensive understanding of these pathways to develop effective targeted therapies [209,216].

Future studies are warranted to elucidate optimal combinations and timing of these therapies and to improve understanding of the underlying mechanisms that enable immune response in the bone niche.

7.3 Extracellular Matrix, Angiogenesis and Other Microenvironment Targets as Potential Therapeutic Targets

The treatment of bone metastases benefits significantly from various microenvironment-targeting strategies that extend beyond traditional approaches, specifically by targeting growth factors like vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGFs), along with stromal reprogramming methods. These strategies aim to alter the tumor-promoting signals within the bone microenvironment, which can facilitate cancerous cell growth and spread.

VEGF plays a crucial role in angiogenesis, which is essential for tumor growth and metastasis. In the context of bone metastases, high levels of VEGF can promote osteolysis and metastasis by enhancing vascular permeability and increasing the delivery of nutrients and growth factors to tumors [217,218]. Multiple studies have examined the efficacy of anti-VEGF therapies like Bevacizumab, noting that while they may reduce angiogenesis and tumor growth, resistance typically arises due to compensatory mechanisms, such as the upregulation of alternative angiogenic pathways, including FGF signaling [219–221]. Therefore, targeting both VEGF and FGF pathways appears to be a promising strategy. Combinatorial approaches that inhibit both pathways have been shown to improve treatment outcomes by preventing adaptive resistance mechanisms that allow tumors to evade single-agent anti-angiogenic therapies [220,221].

FGFs, particularly FGF-2, significantly contribute to tumor progression and metastasis by facilitating angiogenesis and influencing the proliferation and differentiation of stromal cells. Inhibition of FGF signaling has demonstrated potential in reducing metastatic behavior in various cancers, including those with bone involvement [222,223]. For example, FGF-2 participates in the communication between tumor cells and surrounding stroma, promoting an environment conducive to cancer propagation [224,225]. This has prompted research into multi-targeted therapies that address both VEGF and FGF pathways to mitigate the impact of bone metastases [224,226].

Reprogramming the tumor stroma offers another innovative strategy to mitigate metastasis and improve treatment efficacy. The tumor microenvironment in bone metastases is not just a passive backdrop; rather, it actively contributes to tumor progression through various cell types, including fibroblasts and macrophages that are often co-opted by tumors to promote metastasis [227]. For instance, inhibiting the activation of fibroblasts can disrupt their production of pro-tumorigenic factors and extracellular matrix components, thereby hindering metastasis-promoting signaling pathways [224,228].

Macrophages, particularly tumor-associated macrophages (TAMs), play a dual role in cancer; while they can exert anti-tumoral effects, they often become

polarized towards a pro-tumorigenic phenotype, facilitating metastasis and tissue remodeling [227]. Strategies that repolarize these macrophages to a more anti-tumoral phenotype, potentially via immune checkpoint inhibitors in conjunction with stroma-targeting therapies, represent a promising direction in combating bone metastases [205,220,229].

Modifying the extracellular matrix (ECM) can alter the interactions between tumor cells and their microenvironment. Changes in ECM composition and stiffness can directly influence cancer cell behavior, including proliferation, invasion, and migration. By targeting the signaling pathways that regulate these properties within the ECM, researchers can potentially destabilize the supportive niche required for tumor growth and dissemination [219,230,231].

8. Integrated Therapeutic Strategies and Future Perspectives

8.1 Rationale for Combination Therapies

Targeting Multiple Pathways: Cancer cells in the bone microenvironment engage in crosstalk with different cell types, including osteoclasts, osteoblasts, fibroblasts, and immune cells, creating a dynamic and supportive niche for metastasis [232–234]. Effective treatment necessitates strategies that simultaneously disrupt these interactions. For example, targeting the RANK-RANKL pathway involved in osteoclastogenesis alongside inhibiting growth factors like VEGF or FGF can counteract the mechanisms that promote bone degradation and cancer cell proliferation [109,233]. Recent research suggests that dual inhibition can effectively break the crosstalk between cancer cells and bone cells, which is pivotal for tumor progression [234].

Modulation of the Tumor Microenvironment: The bone TME is characterized by immunosuppressive elements, such as macrophages and myeloid-derived suppressor cells (MDSCs), which foster tumor growth and hinder effective immune responses [91,198,235]. Combination therapies that entail immune checkpoint inhibitors alongside TME reprogramming, aimed at altering the profile of infiltrating immune cells, can potentially create a more favorable environment for therapeutic response [111,236]. For instance, the introduction of therapies that convert tumor-associated macrophages (TAMs) from a pro-tumor phenotype to an anti-tumor phenotype may improve the overall immune response against bone metastases [111, 236].

Overcoming Resistance Mechanisms: Resistance to therapy is a significant hurdle in treating bone metastases. Cancer cells can adapt to targeted therapies by activating alternative signaling pathways [222,224,237]. By employing combination therapies that simultaneously inhibit multiple growth factors or signaling pathways, it may be possible to prevent or delay the onset of resistance. For example, studies have shown that targeting both FGFs and VEGF can reduce compensatory angiogenesis that aids in tumor survival and resistance [222–224].

8.2 Personalized Medicine Approaches

Personalized medicine anchored in TME profiling is paving the way for more tailored therapeutic strategies against bone metastases. Assessing the unique characteristics of the TME can inform treatment decisions and improve outcomes. Personalized treatment approaches can utilize molecular profiles from patient tumor samples to identify active signaling pathways and immune cell infiltrates within the TME [238,239]. For example, profiling for the expression of RANKL, PTHrP, or various cytokines can provide insights into the specific requirements of a patient's cancer, allowing for targeted interventions that are best suited to disrupt critical interactions in the bone environment [109,190]. With advancements in screening technologies, identifying biomarkers that predict response to specific therapies is becoming increasingly feasible [120,239]. Such biomarkers may include the expression levels of integrins or immune checkpoint proteins within the TME, which can indicate how responsive a patient's cancer may be to immunotherapies or targeted therapies [111,240]. Adaptive Treatment Strategies: The TME can change dynamically over the course of treatment, especially with the introduction of new therapies. Conducting regular assessments of the TME can allow oncologists to adjust treatment protocols based on the current status of the tumor and the surrounding microenvironment, thus optimizing therapeutic strategies [241,242].

We believe that combination therapies targeting multiple components of the TME, alongside personalized medicine approaches grounded in TME profiling, are necessary strategies for effectively managing bone metastases (Table 1). These approaches not only hold the potential to improve therapeutic efficacy but also to address the complexity and adaptability of the tumor microenvironment.

9. Conclusion and Future Directions

TGF-β is central in the "vicious cycle" of bone metastasis, linking disseminated tumor cells with the bone microenvironment. Released during osteoclastic bone resorption, activated TGF-β functions as both signal and substrate driving epithelial-mesenchymal transition, stimulating osteoclastogenesis, reactivating dormant tumor cells, and suppressing antitumor immunity. This multifaceted role facilitates metastatic colonization and contributes to skeletal complications. Its capacity to orchestrate complex crosstalk among tumor cells, osteoblasts, osteoclasts, stromal fibroblasts, and infiltrating immune populations firmly establishes TGF-β as a master regulator in the pathobiology of bone metastasis. This biological centrality renders TGF-β an attractive yet challenging therapeutic target. A range of strategies—including neutralizing antibodies, ALK5 kinase inhibitors, antisense oligonucleotides,

Table 1. Therapeutic landscape of bone metastasis: current and emerging interventions targeting TGF-β signaling and the skeletal tumor micro-environment.

#	Therapeutic class/example agents	Principal target/mechanism	Development status	Key limitations/considerations
1	TGF-β-neutralizing mAbs (fresolimumab, lerdelimab)	Sequester active TGF-β ligands, blunt SMAD signaling	Phase I–II NCT01401062	Dose-limiting skin & cardiac toxicities; systemic wound-healing concerns
2	ALK5 (TGFBR1) kinase inhibitors (vactosertib, galunisertib)	Block receptor phosphorylation and downstream SMAD2/3	Phase I–II NCT02160106, NCT02423343	Narrow therapeutic window; rebound via parallel pathways
3	Antisense oligos/RNAi (TGF-β2-ASO)	Deplete TGF-β mRNA in tumor & stroma	Early clinical AP12009-P001, NCT03436563	Delivery to bone lesions; off-target immunomodulation
4	Tumor-selective ligand traps/bone-targeted nanoparticles	Localized capture of TGF-β in the metastatic niche	Pre-clinical NCT03834662	Formulation, targeting specificity, scalability
5	RANKL inhibitor (denosumab)	Suppresses osteoclast differentiation & activity	Approved NCT01824342	Does not inhibit tumor growth directly; hypocalcemia risk
6	Bisphosphonates (zoledronic acid, low-dose regimens)	Induce osteoclast apoptosis; reduce SREs	Approved NCT00320710	Renal toxicity, ONJ; no effect on visceral disease
7	Radiopharmaceuticals (radium-223, strontium-89)	α/β-particle emission selectively to bone lesions	Approved/Phase II NCT00699751	Myelosuppression; limited to osteoblastic lesions
8	Immune-checkpoint blockade (anti-PD-1 $\pm$ CTLA-4)	Re-activate exhausted T cells in bone TME	Approved (solid tumors) NCT02834013	Immunosuppressive niche dampens responses; bone-flare phenomena
9	Next-gen checkpoints (TIM-3, LAG-3 mAbs)	Overcome alternative T-cell exhaustion pathways	Phase I NCT05367401	Biomarker selection; combinatorial toxicity

and tumor-selective ligand traps—have demonstrated preclinical and early clinical promise, particularly in combination with immune checkpoint inhibitors, bisphosphonates, or RANKL blockers. However, TGF-β's pleiotropic roles in tissue repair, hematopoiesis, and immune regulation limit the safety of systemic inhibition, raising concerns about impaired fracture healing, cardiotoxicity, and compensatory pro-metastatic signaling. Precision approaches such as patient stratification, pharmacodynamic biomarkers, and targeted delivery platforms will be critical to harness the pathway's therapeutic potential while mitigating off-target effects. Given that TGF-β operates within and is amplified by a dynamic, plastic tumor microenvironment (TME), the single-node blockade is unlikely to be sufficient. Effective strategies must integrate modulation of the immune milieu, osteoclast activity, angiogenic signaling, stromal interactions, and extracellular matrix mechanics to interrupt self-reinforcing loops and counteract resistance. Multi-targeted regimens guided by TME profiling, alongside adaptive clinical trial designs that allow iterative therapeutic adjustment, represent rational paths forward. To advance this field, future research should elucidate the spatiotemporal dynamics of TGF-β activation in human bone metastases using single-cell, spatial-omics, and advanced imaging technologies; define predictive biomarkers, such as SMAD2/3 phosphorylation signatures or TGF-β-responsive gene modules to identify responsive patient subsets; and develop site-specific delivery systems, including bone-targeted nanoparticles or conditionally active biologics, to limit systemic exposure. Moreover, the integration of organ-on-chip platforms, 3D bioprinted bone–TME models, and longitudinal liquid biopsy analyses will be crucial for anticipating resistance mechanisms and refining combination regimens. Embedding mechanistic correlative science into early-phase clinical trials will be essential to link biological insights with patient outcomes, ultimately accelerating translation. By dissecting the complex regulatory network surrounding TGF-β and implementing context-aware therapeutic strategies, we move closer to interventions that mitigate skeletal complications and improve survival and quality of life for patients with metastatic cancer.

Author Contributions

Conceptualization, KSM, and FHB; writing—original draft preparation, KSM, and FHB; writing—review and editing, KSM. Visualization, KSM, and FHB supervision, KSM; project administration, KSM. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare no conflict of interest.

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