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Targeting the Tumor Microenvironment in Osteosarcoma: A Pathway to Overcome Therapeutic Resistance

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Osteosarcoma is the most common primary malignant bone tumor, predominantly affecting children and adolescents. Despite advances in conventional therapies like chemotherapy and surgical resection, the survival rate has remained stagnant due to therapeutic resistance and high rates of metastasis. The tumor microenvironment (TME), a complex network of cellular and non-cellular components, plays a pivotal role in osteosarcoma progression, metastasis, and treatment resistance. Understanding the dynamics within the TME is crucial for developing novel therapeutic strategies that can overcome these challenges. This review explores the key elements of the osteosarcoma TME, including immune cells, endothelial cells, cancer-associated fibroblasts (CAFs), and extracellular matrix (ECM). It examines the roles of cytokines, growth factors, and exosomes secreted by osteosarcoma cells in modifying the TME to foster tumor growth, evade immune surveillance, and promote angiogenesis. Furthermore, the review critically assesses current therapeutic approaches that target TME components, with a focus on disrupting the interactions between the tumor and its microenvironment. This analysis includes a review of clinical trials and preclinical studies evaluating TME-targeting therapies. The findings highlight that the TME actively contributes to osteosarcoma progression by promoting immunosuppression, angiogenesis, and metastasis, while also enhancing resistance to standard treatments such as chemotherapy and immune checkpoint inhibitors. Various therapeutic strategies targeting the TME, such as inhibiting angiogenesis, modulating immune responses, and disrupting CAF and ECM interactions, have shown promise in preclinical models. However, clinical outcomes remain variable, underscoring the complexity of the TME and the need for more comprehensive approaches. Targeting the TME represents a promising pathway to overcome therapeutic resistance in osteosarcoma. While significant progress has been made in understanding the role of the TME in tumor development and resistance mechanisms, further research is required to optimize TME-targeting therapies. A deeper comprehension of the intricate interactions between osteosarcoma cells and their microenvironment may lead to more effective, personalized treatments, improving clinical outcomes for patients with osteosarcoma.

Keywords: Angiogenesis; Chemotherapy Resistance; Metastasis; Osteosarcoma; TME-targeting Therapies; Tumour Microenvironment (TME).

Around 9.8 million fatalities worldwide are attributed to cancer, making it the second most common cause of death for both men and women[1]. Osteosarcoma (OS) is a relatively rare tumor of bone with a worldwide incidence of 3.4 cases per million people per year. For most of the 20th century, 5-year survival rates of 56.31% for classic OS were very low[2].This cancer type

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accounts for approximately 2.4% of all childhood malignancies, with the highest incidence occurring between the ages of 10 and 30. In comparison to the White population, OS is more common in African Americans, Asian/Pacific Islanders, and Hispanics and has a small male predominance. While the precise aetiology of osteosarcoma remains unclear, certain epidemiological risk factors have been associated with an increased likelihood of contracting the illness. Notably, an elevated incidence of osteosarcoma has been associated with Li-Fraumeni syndrome, Rothmund-Tompson syndrome, hereditary retinoblastoma, and Bloom and Werner syndrome. Osteosarcoma is the most prevalent, primary malignant bone pathology and is known for its aggressive nature. Particularly in older populations, other predisposing factors such fibrous dysplasia, Paget's disease of the bone, and radiation exposure are also associated with an elevated risk [3,4]. It's interesting to note that, compared to the general population, osteosarcoma is more commonly recorded in taller people [5]. There are several subtypes of osteosarcoma, the majority of which are high-grade and have aggressive biological behaviour [6]. Results are still not ideal despite advancements in OS treatment [7]. In recent decades, our understanding of cancer has significantly evolved. Cancer is now recognized not merely as a genetic disease but as a complex ecosystem comprising various non-cancerous cells and their extensive interactions within the tumor. While genetic alterations are critical in cancer development and metastasis, they alone are insufficient for the full progression of the disease. The tumor microenvironment (TME) is a highly organized ecosystem where cancer cells are surrounded by diverse non-malignant cell types within a remodeled, vascularized extracellular matrix. This illustrates the intricate complexity of cancer that can be revealed by microscopic analysis of solid tumours. The TME is composed of numerous cell types, including neurones, adipocytes, immunological cells, endothelial cells (ECs), and cancer-associated fibroblasts (CAFs) (Table 1). Initially, host cells within the tumor microenvironment (TME) were considered passive observers of malignancy. However, mechanistic studies, particularly in preclinical tumor models, now suggest that TME cells and the substances they secrete play crucial roles in cancer development, offering promising therapeutic targets[8]. The cellular makeup and functional condition of the TME will be influenced by the organ from which the tumour originates, the inherent traits of cancer cells, the tumor's stage, and the patient's features. Different cells within the TME can either be tumour supportive or tumour suppressing. Osteosarcoma progression, metastasis, angiogenesis, hypoxia-induced resistance, and treatment resistance are all significantly influenced by the tumour microenvironment (TME). The TME is made up of many cell types that interact intricately with cancer cells, such as immune cells, fibroblasts, endothelial cells, and components of the extracellular matrix. These interactions present a number of difficulties for existing treatments, including radiation, chemotherapy, and newer forms of therapy like immunotherapy. Due to its role in drug resistance, immunosuppression, and tumour heterogeneity, the TME presents serious treatment-related problems. For the purpose of creating novel treatment approaches, such as combination therapies that target both the tumour cells and the surrounding microenvironment, a deeper comprehension of the TME's function in cancer biology is vital. Getting beyond these obstacles will be essential to enhancing treatment results and stopping the spread of cancer. This review examines strategies to target the tumor microenvironment (TME) in osteosarcoma to overcome therapeutic resistance and enhance treatment efficacy. The TME comprising immune cells, blood vessels, fibroblasts, and metabolic factors supports tumor growth and resistance to therapy. By focusing on approaches like immune checkpoint inhibitors, anti-angiogenic agents, fibroblast disruptors, and exosome-based delivery, this review outlines a multi-faceted approach to improve osteosarcoma treatment outcomes. Hence proved, previous studies targeting the tumor microenvironment (TME) in osteosarcoma have shown promising results. Immune checkpoint inhibitors like anti-PD-1 have enhanced immune responses , while anti-angiogenic agents (e.g., bevacizumab) and multi-kinase inhibitors (e.g., sorafenib) have reduced tumor vascularization . Drugs targeting cancer-associated fibroblasts, like trabectedin, have improved chemotherapy sensitivity. Additionally, hypoxia-activated drugs and exosome inhibitors have helped reduce metastasis and increase drug

delivery precision. This review seeks to explore the relationships between osteosarcoma cells and the TME that lead to cancer growth, metastasis, and treatment failure in addition to identifying promising therapeutic targets and innovative strategies to improve treatment outcomes[9].

MATERIALS AND METHODS

A comprehensive literature search was conducted using PubMed, Scopus, and Web of Science to identify peer-reviewed articles on targeting the tumor microenvironment (TME) in osteosarcoma. Included studies were preclinical and clinical research addressing TME-related therapeutic resistance mechanisms or therapies targeting TME components, such as immune cells, cytokines, or the extracellular matrix. Articles on other cancers, studies with inadequate data, or review articles lacking original evidence were excluded. Key findings were categorised by TME components, such as immune suppression, angiogenesis, and ECM remodelling, and their effects on treatment outcomes were examined by data synthesis.

Components of the Tumor Microenvironment in Osteosarcoma

Cancer-Associated Fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) have a variety of protumorigenic roles in the tumour microenvironment. Clinical translation of these preclinical findings which point to the possibility of reducing, eliminating, or reprogramming CAFs has not yet taken place. Undoubtedly, a deeper comprehension of these cells and their roles will enhance cancer therapies. Nearly every organ and healthy tissue contains fibroblasts, which aid in wound healing by promoting fibrosis and inflammation. CAFs are mesenchymal-lineage activated fibroblasts linked to cancer that promote inflammation and fibrosis around tumours. The lack of lineage markers for haematopoietic, endothelial, and epithelial cells, as well as their shape and correlation with cancer cells, characterize CAFs [14]. In addition to playing important roles in immune modulation, angiogenesis, and extracellular matrix remodelling of the tumour, CAFs also generate and maintain cancer stem cells, which in turn promotes therapy resistance. Numerous cell progenitors, which can differ between tissues, can give rise to CAFs. Not all CAFs have their origins clearly and completely explained. Although the CAF phenotype differs within and between tumour types, the change to CAF, regardless of its cause, is almost invariably irreversible.Although they can also evolve into CAFs, mesenchymal stromal cells, also known as mesenchymal stem cells, are mostly derived from local resident fibroblast populations.MSCs can differentiate into osteoblasts, chondrocytes, and adipocytes and display a similar, less abundant set of surface markers [16]. CAFs and resting fibroblasts from normal tissues differ significantly in a few key ways. CAFs have a spindle-shaped morphology, branching cytoplasm, and indented nuclei. Often, they are bigger than fibroblasts that are at rest. This, however, is the main functional distinction between the two. The ability of CAFs to secrete, migrate, and proliferate has enhanced. CAFs produce more extracellular matrix components, such as tenascin, periostin (POSTN), and secreted protein rich in cysteine (SPARC), than untransformed fibroblasts because of their increased metabolic activity. Increased synthesis and frequently a more rigid and contractile pattern of collagen deposition are characteristics of aberrant collagen production by CAFs [17]. Tumours are diverse ecosystems that vary in both function and geography.The variety of origins that might give rise to CAFs complicates their phenotypic, gene expression, and functions. While numerous investigations have also looked at these cells in other human tumour types, the majority of research on different CAF subpopulations at the single cell level has been carried out in the context of pancreatic cancer in humans. In human pancreatic cancer, the expression of á-SMA and IL-6 defines at least two primary CAF phenotypes, which are examples of some of these classes. iCAFs, a myofibroblastic Cancer – Associated Fibroblasts (myCAF) population with low á-SMA expression and high levels of IL-6 and IL-11 secretion, are more inflammatory in nature, matrix-secreting, TGF-â sensitive, high in á-SMA and low in cytokines (e.g.,IL-6,IL-11)[18].

Immune Cells

Myeloid-derived suppressor cells (MDSCs), T-regulatory cells (Tregs), and tumor-associated macrophages (TAMs) collaborate to produce an immunosuppressive tumour

microenvironment (TME) that aids in the survival and dissemination of cancer. TAMs secrete cytokines, like IL-10 and TGF-â, that promote Treg growth, inhibit T cell activity, and contribute in the growth of tumours. By secreting immunosuppressive cytokines, consuming IL-2, and blocking effector T cells with the help of molecules like CTLA-4, tregs impair anti-tumor immune responses. By generating reactive oxygen species, consuming less essential nutrients, and upregulating immunological checkpoints such as PD-L1, MDSCs further inhibit T cell activity. Collectively, these cells facilitate tumour immune evasion, reduce the immune response, and increase resistance to treatments like chemotherapy and immunotherapy [19].

Extracellular Matrix (ECM)

The extracellular matrix (ECM) facilitates the motility, invasion, and metastasis of osteosarcoma cells by providing structural support and biochemical cues that stimulate cancer growth. Extracellular matrix (ECM) compositional changes and remodelling enzymes, such as matrix metalloproteinases (MMPs), degrade the ECM in osteosarcoma, allowing cancer cells to proliferate. In addition, the ECM stimulates signalling pathways that enhance cell invasion, motility, and survival through interactions with integrins and other cell surface receptors. Furthermore, the extracellular matrix (ECM) hardens and rearranges to promote cancer cell infiltration into neighbouring tissues, permitting metastasis to far-off organs like the lungs. The aggressiveness and treatment resistance of osteosarcoma are influenced by these ECM alterations[20].

Hypoxia and Its Impact on Osteosarcoma Progression

One histological feature that is frequently observed on biopsies is that bone cancer is a highly necrotic tumour even at the time of diagnosis.The rapid and uncontrolled expanding proliferation of cancer cells, which are skilled at producing an aberrant neo angiogenesis [21] and hypoxic microenvironment [22], may be the cause of this necrosis. OTS is distinguished by the assessment of the necrotic rate upon surgical tumour resection following a neo adjuvant multi chemotherapy approach, in addition to the discovery of tumour necrosis during biopsy. The endpoint of extreme persistent hypoxia is thought to be tumour necrosis. Because it induces hypoxia-related indicators, this necrosis can alter metabolic responses and exacerbate oxidative stress. On the other hand, hypoxia may also cause apoptosis and necrosis to be inhibited during the course of cancer treatment and progression [23]. Hypoxia has the ability to cause necrosis in the tumor's core. Thus, the hypoxia-specific tumour microenvironment may be significant for the development, oncogenesis, and treatment of OTS. Consequently, in order to comprehend their function and determine their druggability in OTS, we attempt to unravel the normoxic/hypoxic pathways in healthy tissues, bones, and osteosarcomas.

RESULTS

Hypoxic rHypoxic regions within the tumor microenvironment promote osteosarcoma survival and metastasis by inducing Hypoxia Inducible Factors (HIFs)

The marrow milieu surrounding bone contains growth factors, cytokines, blood supplies, and tumor-supporting cells such as T cells, stromal cells, and macrophages. In the growth and remodelling of the skeleton, osteoblasts and osteoclasts are essential. Two distinct processes intra membranous or endochondral ossification can result in the formation of bone. Flat bones are the exclusive domain of the intra membranous bone development. It starts off as mesenchymal cells, which go on to become osteoblasts for the skull. It originates from a chondrocyte anlage, which is replaced by bones in other parts of the flat skeleton [24].Endochondral bone production occurs in three stages, with physioxia and hypoxia playing crucial roles. In the first stage, mesenchymal cells condense and differentiate into chondrocytes, which ultimately form growth plates. These chondrocytes proliferate significantly in the growth plates, creating columnar layers.At the distal end of these layers, cells cease proliferation, exit the cell cycle, and develop into hypertrophic chondrocytes associated with mineralization.[24,25]. The growth plate itself is a specialized form of mesenchymal tissue containing hypoxic and avascular regions[26]. To overcome this challenging environment, chondrocytes need to synthesise VEGF-A and produce HIF-1á in order to initiate the angiogenic switch, which permits cartilage

to be replaced by bone [25]. The stabilisation of HIF-2á, which counteracts HIF-1á activity, and the production of pVHL, which is controlled by HIF-1á, are connected to this overexpression [27]. In actuality, throughout the endochondral process, the HIF signalling system is crucial for controlling the vascular and osteoblastic niches. It is thought that osteoblast activity, quantity, and bone formation are positively regulated by HIF-1á. It hastens bone macrophage senescence by encouraging non-oxidative glycolysis in osteoblasts and postponing osteoclastogenesis [25]. Thus, it may be inferred that HIF-1á primarily influences osteoclast-mediated bone resorption while having

Table 1. Major cellular and non-cellular components of the TME (Original)

Type of Cell	TME Function	Ref
	Adaptive immune cells	
$CD4^+$ T cells	CD4+ helper T cells primarily influence CD8+ T cell responses and other immune cells. In cancer, CD4+ T cells play dual roles: The subtype exhibits anti-tumor activity by producing IFNã and TNF-á, directly killing cancer cells and supporting CD8+ T cells and B cells, while the the subtype secretes pro-tumoral and anti-inflammatory mediators. Emerging evidence suggests that CD4+ T cells are crucial for the effectiveness of Immune Checkpoint Blockade (ICB).	10
$CD8^+$ T cells	CD8+ T cells are key effectors in tumor immune response, recognizing cancer cells via TCR binding to MHC-peptide complexes. After engagement, they induce apoptosis through granzyme, perforin, or FASL-FAS pathways. In tumors, CD8+ T cells often appear exhausted or dysfunctional. Immune checkpoint inhibition aims to reactivate these CD8+ T cells to fight cancer.	11
	Myeloid immune cells	
Macrophages	Tumor-Associated Macrophages (TAMs) are versatile, with both pro- and anti-tumorigenic roles. Derived from bone marrow or yolk sac, TAMs exist in various subtypes within tumors. While they can directly phagocytose cancer cells and stimulate anti-tumor immunity, TAMs also promote angiogenesis, immunosuppression, metastasis, and treatment resistance.	12
	Immune cells bridging the gap between innate and adaptive immunity	
NK cells	Natural Killer (NK) cells are cytotoxic innate lymphoid cells that detect and destroy stressed cells lacking MHC class I. Higher levels of circulating and intratumoral NK cells are associated with better cancer survival. While NK cells have strong anti-tumor activity, tumors evade them through immune-suppressive myeloid cells, Tregs, and inhibitory receptor overexpression. NK cell-based therapies or activation of endogenous NK cells are emerging as promising immunotherapeutic strategies.	13
	Stromal cells and matrix	
Cancer-associated fibroblasts	Cancer-associated fibroblasts (CAFs) are key components of the tumor stroma, consisting of diverse, functionally flexible subtypes. In the tumor microenvironment (TME), CAFs play complex roles, synthesizing and remodeling the ECM, affecting its mechanical properties and influencing cancer cell behavior. They promote angiogenesis, modulate the immune response, and aid in immune evasion by tumors.	14
	Vascular cells	
Blood vascular endothelial cells	Endothelial cells (ECs) line all blood vessels, but tumor ECs differ from normal ones. Tumor ECs are highly diverse, with reduced adhesion molecules that weaken barrier function and increased inhibitory immune checkpoint molecules, promoting immunosuppression. They regulate fluid, oxygen, protein, and cell flow in the tumor environment.	15

minimal impact on osteoclast differentiation [28]. To give the macrophages adaptive support during bone resorption, it speeds up glycolytic and mitochondrial metabolism and upregulates the expression of cytokines that may regulate the process of differentiation [29,30]. On the other hand, HIF-2á could be seen as a negative regulator of the formation of bone mass that influences the osteoblast lineage directly [25]. Similarly, MIF (Macrophage Migration Inhibitory Factor) appears to be p53-dependent in its regulation of HIF-1á activity.The pathological hypoxia and physiological oxygen tension that arise during cancer processes may present a favourable environment for the homing, initiation, and proliferation of tumour cells in the context of a hypoxic growth plate.

The role of hypoxia in mediating resistance to chemotherapy and radiotherapy

It seems that hypoxia plays a critical role in both local and remote OTS scenarios. As was already known, the local environment of growth plates, which is permissive to fluctuations in oxygen levels, is where osteosarcoma cells often develop. The onset and progression of osteosarcoma appear to be correlated with changes in the hypoxia biomarkers. Numerous studies have demonstrated correlations between the poor prognosis of OTS and certain biomarkers, such as mTOR, HIFs, or CA IX (Carbonic Anhydrase IX) [26-28]. They are emphasizing how hypoxia is the primary factor causing OTS cancer growth, treatment resistance, and propensity for metastasis. Using both tumour collections and *in vitro* or *in vivo* preclinical models, it was possible to illustrate the concept of hypoxia in osteosarcomas through an examination of the study materials. But the primary hypoxia-related marker associated with OTS has been HIF-1á, which is often overexpressed in locally aggressive and metastatic OTS [28]. It is possible to explain the relationship between the overexpression of GLUT-1 (Glucose Transporter 1), CA IX, or VEGF/VEGFR and the overall enhancement of hypoxic pathways from the membrane to the nucleus as well as the rise in intra-tumor microvessel density [29]. At that time, HIF-1á was primarily identified as a key factor in the modification of the tumour microenvironment. Although HIF-2á has not received as much research as its homologues, it appears to have a role in OTS stemness characteristics promotion and apoptosis [30]. Consequently, it was discovered that HIF-2á was primarily a significant driver in OTS cells that experienced a certain metabolic change. An OTS cell and cancer microenvironment effect was combined by mTOR, which was generally directly associated to autophagic processes and linked to Pi3K/AKT upstream signalling induced by distinct tyrosine kinase receptors [31]. However, since hypoxia is present from the beginning of OTS cells, it is still unclear, for example, how precisely all those indicators interact during OTS progression and metastatic propension. While more research on OTS is required, it is now understood that the sporadically involved hypoxia signalling pathways in various cancer types can be explained by a balanced HIF-1á/HIF-2á and variations in both markers' expression [19]. Recent research has linked increased genomic instability in cancer cells, particularly in osteosarcomas, to intra-tumor hypoxia. Osteosarcomas often display elevated levels of chromosomal breakage and chromothripsis, a phenomenon involving extensive chromosome rearrangement and fragmentation within the tumor.[32]. A poor prognosis is associated with this genomic instability and complexity, which are often accompanied in these tumour types with a high dysregulation of microRNAs following hypoxia. It has likely been demonstrated that miRNA-133a contributes to chromosomal dysregulation and the advancement of osteosarcoma in OTS [33].

Interaction Between the Tumor Microenvironment and Osteosarcoma Cells

The interaction between these cancer cells and the tumour microenvironment (TME) is essential for osteosarcoma growth, invasion, and treatment resistance.Pro-inflammatory cytokines, such IL-6 and IL-10, attract tumor-associated macrophages (TAMs) and T-regulatory cells (Tregs) into an immunosuppressive environment and aid in immune evasion. Osteosarcoma cells release growth factors including VEGF and TGF-â, which promote angiogenesis and cancer survival. Furthermore, exosomes carrying proteins and microRNAs that affect stromal cells and encourage angiogenesis, metastasis, and ECM remodelling are released by osteosarcoma cells. Osteosarcoma cells and the TME interact in a way that increases tumour aggressiveness and promotes treatment resistance [34]. Tumour microenvironment (TME) composition

and behaviour are modified by osteosarcoma cells to facilitate tumour growth, invasion, and resistance to treatment. In order to promote angiogenesis and guarantee a sufficient blood supply for oxygen and nourishment, they produce growth factors including VEGF and TGF-â. Additionally, the extracellular matrix (ECM) is broken down by matrix metalloproteinases (MMPs) secreted by osteosarcoma cells, which promotes cell migration and invasion of nearby tissues. They also produce cytokines like IL-6 and IL-10, which attract and activate immune-suppressive cells, including T-regulatory cells (Tregs) and tumor-associated macrophages (TAMs), thereby reducing the tumor's ability to elicit an effective immune response. Osteosarcoma cells also communicate with one another through exosomes, which carry proteins, microRNAs, and other substances that impact distant organs and surrounding stromal cells, promoting metastasis and treatment resistance.Osteosarcoma cells modify the TME via these pathways in order to improve their chances of surviving, proliferating, and spreading [35].

Influence of the Tumor Microenvironment on Therapeutic Resistance

TME contributes to chemotherapy and radiotherapy resistance in osteosarcoma

The TME is made up of different cell types (fibroblasts, endothelial cells, immune cells), extracellular elements (chemokines, cytokines, hormones, and extracellular matrix) that surround and are fed by the vasculature, and different physical and chemical elements (an acidic environment and hypoxia). The TME is necessary for metastasis, carcinogenesis, and tumour development. The tumour microenvironment (TME) has a major impact on the initiation and maintenance of cancer hallmarks, such as promoting angiogenesis, maintaining proliferative signalling, preventing cell death, and initiating invasion and metastasis [36]. The effectiveness of treatment is also significantly impacted by the TME. The result of continuous interactions between the surrounding matrix and cancer cells is TME-reduced multidrug resistance. Compared to non-tumor cells in the TME, which are more genetically stable and responsive to stimulation, cancer cells are more likely to exhibit chemo resistance due to their genomic instability. The idea that efforts aimed at addressing TME elements or their signaling pathways could result in therapeutic breakthroughs for cancer patients is raised by the finding that cancer growth and treatment resistance are strongly associated with the TME.

Hypoxic TME and chemoresistance in OS

Because of their hypermetabolism, aberrant growth, and excessive oxygen consumption, tumour cells usually exist in a hypoxic environment [37]. There is now strong evidence linking hypoxia-inducible factors (HIFs) to medication resistance [38]. Numerous genes involved in angiogenesis, glycolysis, and erythropoiesis can be induced to express themselves during hypoxia by HIFs produced for hypoxic adaptation. They can also bring back oxygen equilibrium through transcription and epigenetics [39]. Hypoxia can, in fact, result in an acidic environment; the classic example of this is the Warburg effect, in which cancer cells favour using glycolysis as a fuel source. H+-ATPases, Na+-H+ exchangers, and HCO3-transporters are able to transfer the acidosis from an intracellular to an external environment [40].

Moreover, the rapid growth of tumour and their abnormal vascular patterns accelerate the build-up of acid, causing cancer cells to have an intracellular pH greater than 7.4 and an exterior pH of 6.6-7.1. By contrast, normal cells have an internal pH of 7.2 and an external pH of about 7.4 [41].

Tumor-associated macrophages modulate chemoresistance in OS

Important elements of the tumour microenvironment (TME), Tumor-Associated Macrophages (TAMs) typically exhibit tumor-suppressive characteristics and regulate treatment response. Rather than being founded in resident macrophages that proliferate within tumours, TAMs in solid tumours are rooted in circulating monocytes. Bone marrow monocytes have the ability to penetrate tumours through the bloodstream and then develop into macrophages. Type M1 and type M2 macrophages are distinguished by the degree of polarisation. M2 macrophages are activated by cytokines like interleukin (IL)-4, IL-10, and IL-13, whereas M1 macrophages are driven to differentiate by cytokines like interferon-gamma (IFNg) [42]. In a similar vein, M1 macrophages are typically thought to combat cancer, but M2 macrophages aid in the development of

cancer [43]. As a matter of fact, the TME regulates TAM functional polarisation in a significant way [44]. Drug resistance in OS mediated by angiogenesis. Angiogenesis is a hallmark of cancer and a highly adaptable process that is essential to tumour growth, metastasis, and medication resistance. Many processes, including blood vessel development, smooth muscle cell recruitment, endothelial cell migration, differentiation, and proliferation, are associated with angiogenesis [45]. A malignant vascular network that is marked by dilated, convoluted, and hyperpermeable vessels can arise from tumours that exhibit an unbalanced balance between pro- and anti-angiogenic signals. This can lead to spatiotemporal heterogeneity in the oxygenation and blood flow of the tumour or an increase in the interstitial fluid pressure of the tumour [46]. Furthermore, abnormal bone homeostasis can be brought on by dysregulation of angiogenic and angiocrine processes [47]. The compromised effectiveness of chemotherapy, radiation, and immunotherapy is a clear indication of the physiological effects of these vascular anomalies and the milieu that follows, which promotes tumour growth [46]. Apart from its involvement in acidity, hypoxia, and increased IFP in drug resistance, angiogenesis also limits the absorption of anticancer drugs due to the abnormal vascular architecture of OS [48]. Anticancer drug distribution is uneven because chemotherapeutics must penetrate tumour tissues and blood vessel walls in order to destroy cancer cells. As a result, some target tumour cells that are close to tumour blood arteries are exposed to a potentially fatal dose of the cytotoxic chemical [49]. As a result, the drug's killing power is restricted. The role of CAFs and immune cells in mediating drug resistance through signaling pathways. Adhesion, growth, proliferation, motility, and survival of cells are all regulated by PI3K/Akt, one of the most important intracellular signal transduction pathways [50]. A growing body of research [51] has demonstrated that human cancer, including OS, is associated with aberrant expression of PI3K/Akt signalling pathway components. Immuno staining study has revealed a substantial and significant correlation between PI3K/Akt signalling and a poor prognosis in primary OS cases [52]. Moreover, Akt activity has a substantial correlation with lung metastasis. Several investigations have exhibited how aberrant expression of proteins might trigger the PI3K/Akt signalling pathway, contributing to the pathogenesis of OS [53-57]. A glycoprotein on the surface called intracellular adhesion molecule-1 (ICAM-1) facilitates cell-ECM interaction and encourages metastasis in malignancies. The Fractalkine/CX3CR1 axis can be used to increase ICAM-1 expression, which will help OS cell motility. The mechanism is mediated by the PI3K/Akt/NF-êB signalling pathway. The Fractalkine/CX3CR1 axis has the capacity to phosphorylate Akt through PI3K within the PI3K/Akt/NF-êB cascade. NF-êB may then be further activated as a result. In the end, NF-êB plays the role of a transcription factor, helping to produce ICAM-1 [53]. The IL-8/CXCR1 axis can directly activate Akt signalling to improve OS resistance to Anoikis [58]. Tumor-suppressing STF cDNA 3 (TSSC3), an apoptosis-related imprinted gene and a prognostic marker for OS patients, can activate autophagy in OS and disrupt the src-dependent PI3K/Akt/mTOR signalling pathway to inhibit cell migration and invasion in vitro and in vivo [54]. In addition to being essential for maintaining the extracellular matrix's formation, overexpression of fibrin-4 initiates the PI3K/Akt/mTOR signalling pathway, which promotes OS cell invasion and metastasis [55]. In the meantime, EMT, a critical biological process in cancer cell metastasis, is accelerated by Fibulin-4 overexpression [55]. Increased expression of GPNMB stimulates the PI3K/Akt/mTOR signalling pathway and facilitates the development and metastasis of OS cells [56]. Furthermore, transcription factors affect the activation of PI3K/Akt. For instance, overexpression of the zinc finger transcription factor ZIC2 can promote OS cell motility, invasion, and survival by activating PI3K/Akt[57].

Targeting the Tumor Microenvironment in Osteosarcoma Therapy

Anti-angiogenic Therapy

Over the past ten years, a number of Phase I/II clinical trials have been carried out, mostly involving advanced-stage OS, to treat neovascularization in OS [59]. Clinical trials involving anti-angiogenic drugs that target VEGFRs, like sorafenib alone [60] or in combination with everolimus, a mTOR2 inhibitor [61], have shown the greatest promise. Treatment advantages were observed in the Italian Sarcoma Group trials on recurrent OS, with 46–47% of patients showing a

4-6 months increase in progression free survival. However, in OS patients with resectable and localised OS, the combination of pre and post-operative chemotherapies with the well-known anti-VEGF antibody bevacizumab did not improve patient outcomes or increase the proportion of patients who reacted favourably to neo-adjuvant therapy. However, the therapy may cause problems with wound healing following surgery [62]. In a spinal OS instance, the combination of sorafenib and denosumab, an antibody that targets RANKL found in the osteoid matrix, led to a successful metabolic tumour regression [63]. Preclinical research has recently investigated targeting VEGFR-2, a VEGF receptor expressed mostly on angiogenic arteries but also on OS cancer cells, as a potential anti-angiogenic treatment approach [64]. According to findings, VEGFR2/STAT3/BCL2 signalling links the highly selective VEGFR-2 inhibitor apatinib to direct anti-tumoral efficaciousness [64]. It has also been demonstrated that using the monoclonal antibody ramucirumab to target VEGFR-2 has anti-angiogenic effect in vitro [65]. Surprisingly, even with the addition of doxorubucin-based cytotoxic treatment, anti-mouse Vegfr-2 antibody infusion did not change tumour growth in preclinical OS paediatric cancer models.This implies that, although it is challenging to accomplish effectively, targeting both the tumour and the vascular microenvironment is essential in OS.

Immune Modulation

The TME demonstrates a wide range of immune cell compositions found in various cancer types. Some cancers show very few indications of inflammation, while other tumours include a large number of immune cells within or around them [66]. Cells from both arms of the immune system make up the TME, and depending on the chemical cues it contains, the same type of immune cell may either promote or hinder the growth of tumours [67].Cancer cells are surrounded by a persistent overexpression of inflammatory mediators, which makes it harder for the immune system to recognise and eradicate aberrant cells i.e., immune cells develop a tolerance to cancer cells [68]. Several tactics could be used to slow the growth of the tumour due to the immune system's involvement in cancer: (1) focussing on chronic inflammation or pro-tumorigenic factors supplied by adaptive immune cells (2) blocking macrophage differentiation into the pro-tumoral phenotype (TAMs) (3) inhibiting macrophage recruitment into tumour tissues (4) inducing anti-tumoral activity to prevent the development of cancer or a poor prognosis for the patient in the event that a tumour has already grown [69].

Stromal Targeting

In the extracellular matrix (ECM), stromal cells must move, multiply, and produce in order for tissue inside an organ to function correctly [70].Mesenchymal stromal cells are a highly varied population of progenitor cells with a range of origins that are present in most adult human tissues and play a significant role in the genesis of malignancies. The ECM remodelling characteristics of mesenchymal stromal cells include their capacity to develop into non hematopoietic cells and take part in collagen turnover. Furthermore, they have a role in immune response regulation, tissue regeneration, and repair [70,71]. Mesenchymal stromal cells are drawn to TME's wound like structure in an effort to repair injured tissue [70]. The response of mesenchymal stromal cells to external stimuli can result in an inflammatory phenotype of macrophages that either promotes or prevents the growth of tumours [70-72]. For instance, proangiogenic and immune suppressive substances (e.g., EGF, PDGF, fibroblast growth factor 2, FGF-2, VEGF, IL-6, and IL-10) are created when TNF, IL-1, IFN-ã, and hypoxic conditions are present in the TME, which promotes tumour growth [71].

Emerging Therapies and Clinical Trials

Small molecule inhibitors, immunotherapies, and medications that interfere with the tumor stroma interaction are some of the novel treatment strategies that target the TME. These strategies also concentrate on interfering with important interactions that promote tumour growth and resistance. While VEGF inhibitors restrict angiogenesis and cut off the tumor's blood supply, small molecule inhibitors such as MMP inhibitors target matrix remodelling enzymes to prevent tumour invasion and metastasis. Immunotherapies, such as immune checkpoint inhibitors (anti-PD-1/PD-L1), function to reawaken the immune system and break through the immunosuppressive TME, increasing the ability of T cells to fight cancer cells. Furthermore, medications that interfere with the tumor-stroma interaction like TGF-â blockers or CAF inhibitors hinder the extracellular matrix's components and

cancer-associated fibroblasts' ability to sustain the tumour, decreasing the tumor's structural and biochemical support system. These therapies collectively target the TME to inhibit tumor progression and improve treatment outcomes. Several drugs that target components of the tumour microenvironment (TME) in osteosarcoma are being studied in ongoing clinical studies in an effort to increase therapeutic success. Immune checkpoint drugs, such nivolumab (anti-PD-1) and pembrolizumab, are being tested in order to reverse TME-mediated immunosuppression and reactivate T cells to combat tumours. Clinical trials are also being conducted on angiogenesis inhibitors, which include sunitinib and bevacizumab (anti-VEGF), to stop the creation of new blood vessels that are necessary for the growth of tumours. Other strategies involve using MMP inhibitors to target cancer-associated fibroblasts (CAFs) and ECM remodelling enzymes, as well as investigating TGF-â blockers to sabotage the tumor-stroma relationship. The TME is a crucial target for novel osteosarcoma treatments, as several trials are combining these medicines with traditional chemotherapy to improve outcomes.

Future Directions and Challenges

Osteosarcoma may respond better to treatment if treatments are developed that alter the tumour microenvironment (TME). Targeting the Extra Cellular Matrix (ECM), Tumor-Associated Macrophages (TAMs), and Cancer-Associated Fibroblasts (CAFs) might disturb the milieu that promotes tumour growth, invasion, and immune evasion. Chemotherapy, immunotherapy, and targeted therapies can be more effective when strategies such as switching TAMs from a pro-tumor (M2) to an anti-tumor (M1) phenotype, inhibiting MMPs to prevent ECM disintegration, and limiting TGF-â to decrease stromal support are combined. These strategies seek to modify the TME in order to decrease drug resistance, enhance immune system identification of cancer cells, and ultimately make osteosarcoma more susceptible to conventional therapies. Because the tumour microenvironment (TME) is complicated and overlaps with normal physiological processes, developing medicines that specifically target the TME without damaging healthy tissues presents substantial obstacles. Because growth factors, cytokines, and extracellular matrix (ECM) proteins are among the components of the TME that are also present in normal tissues, it is difficult to create therapeutics that specifically disrupt the relationships that fuel tumour growth without compromising healthy cells. Targeting immune cells such as T-regulatory cells (Tregs) or tumor-associated macrophages (TAMs) may also compromise immune function normally, perhaps resulting in autoimmune illness or inadvertent immune suppression. Furthermore, the dynamic and varied character of the TME across different tumor kinds and stages hampers the development of universal therapeutics. Improved biomarkers and delivery methods are needed to achieve accurate targeting in order to reduce off-target effects and increase TME specificity. Tumour microenvironment (TME) research in osteosarcoma is undergoing a revolution thanks to the introduction of sophisticated models like as organoids and 3D tumour cultures, which provide more physiologically realistic systems for understanding tumor stroma interactions and treatment responses. Researchers can examine how osteosarcoma cells interact with their surroundings in a more realistic setting by using 3D models and organoids, which simulate the complex architecture, cellular variety, and ECM composition of real tumours, in contrast to typical 2D cell cultures. These models aid in the investigation of tumour invasion, migration, and immune evasion. They also facilitate the assessment of the effects of TME-targeting medicines on the course of osteosarcoma.

DISCUSSION

The reviewed studies reveal that the osteosarcoma Tumor MicroEnvironment (TME) is highly immunosuppressive, with Tumor-Associated Macrophages (TAMs), regulatory T cells (Tregs), and Myeloid-Derived Suppressor Cells (MDSCs) promoting immune evasion and resistance to therapy. Targeting these cells using CSF-1R inhibitors and anti-CTLA-4 therapies has shown promise in enhancing anti-tumor immunity and sensitizing cells to chemotherapy. Additionally, angiogenesis, driven by VEGF, contributes to resistance, but anti-VEGF therapies like bevacizumab show mixed results, suggesting the need for multi-pathway inhibition. The extracellular matrix (ECM) also acts as a

barrier to drug penetration, and its disruption using MMP inhibitors or enzymatic approaches improves chemotherapy delivery. However, targeting the ECM selectively remains challenging. Future research should focus on combining immune checkpoint inhibitors, anti-angiogenic therapies, and ECM-targeting strategies, with nanotechnology-based drug delivery offering a potential solution to improve therapeutic efficacy in osteosarcoma.

CONCLUSION

One important factor influencing the development, metastasis, and treatment resistance of osteosarcoma is the Tumour MicroEnvironment (TME). Growth factors, cytokines, and exosomes secreted by osteosarcoma cells interact with the TME to form a microenvironment that facilitates tumour survival and immune evasion. The TME continues to be a major obstacle to effective therapy, despite advancements in conventional treatments, and it also plays a role in drug resistance and tumour recurrence. These obstacles may be addressed by employing cutting-edge treatment approaches that target certain TME constituents, such as immune cells, cancer-associated fibroblasts, and ECM remodelling enzymes. To find more efficient, focused treatments, additional investigation into the processes of TME modulation and the creation of sophisticated preclinical models, such as organoids and 3D cultures, are necessary. By addressing the supportive role of the TME, future treatments may improve outcomes for osteosarcoma patients and reduce therapeutic resistance.

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