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Review

Targeting Metabolic Checkpoints: Remodeling the Immunosuppressive Microenvironment in Osteosarcoma

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Abstract

Osteosarcoma is a malignant bone tumor that frequently occurs in adolescents. Its highly immunosuppressive microenvironment is the key factor leading to the failure of conventional therapies and resistance to immunotherapy. Tumor cells competitively consume key nutrients and secrete metabolites such as lactic acid through metabolic reprogramming (such as the Warburg effect), creating an "acidic, hungry" environment that is unfavorable for the survival and function of effector immune cells, while promoting the activation of immunosuppressive populations such as regulatory T cells. This review aims to systematically explain how this metabolic crosstalk drives immunosuppression and explore the latest strategies for targeting key "metabolic checkpoints" to disrupt this harmful dialogue and reshape the immune microenvironment. Finally, we propose a combined strategy that combines metabolic regulators with existing immunotherapies as a future direction for reversing osteosarcoma immunosuppression and improving patient prognosis, and also discuss the challenges in this field.

Keywords: osteosarcoma; tumor microenvironment; metabolic reprogramming; immunotherapy; glycolysis; exosomes

1. Introduction

Osteosarcoma, the most common primary malignant bone tumor [1], stands out among human malignancies due to its distinct biological and clinical characteristics, which fundamentally differ from the more prevalent epithelial-derived cancers (e.g., lung, breast, colorectal cancers). This divergence originates from its mesenchymal cell origin, leading to unique genomic, immunological, and metabolic landscapes that contribute to its specific therapeutic challenges [2,3].

Unlike epithelial cancers, which often arise from specific oncogenic driver mutations and exhibit "immune-hot" or "immune-inflamed" phenotypes responsive to immunotherapy, osteosarcoma presents a more complex and less immunogenic profile [4]. The table below summarizes the key distinctions (Table 1, Ref. [2,4,5,6,7,8,9,10]).

These intrinsic differences underpin the profound resistance of osteosarcoma to conventional targeted therapies and modern immunotherapies. Unlike the remarkable success of PD-1/PD-L1 inhibitors in certain epithelial cancers, the objective response rate in osteosarcoma clinical trials remains disappointingly low [11,12]. This predicament has prompted researchers to delve deeper into the underlying resistance mechanisms. The current consensus is that the highly immunosuppressive tumor microenvironment within osteosarcoma is a key barrier that weakens the efficacy of immunotherapy [13,14]. This microenvironment is not an "immune desert", but is filled with dysfunctional immune cells, for example: infiltrating cytotoxic CD8+ T cells often present an "exhausted" phenotype, with severely impaired killing function. Regulatory T cells with immunosuppressive function, myeloid-derived suppressor cells, and M2-type tumor-associated macrophages are abnormally enriched, collectively forming a "firewall" to protect tumors [15,16].

So what dominates the establishment and maintenance of this immunosuppressive state? More and more evidence is pointing the finger at metabolic reprogramming of tumor cells—a key role that is increasingly prominent in osteosarcoma. Osteosarcoma cells exhibit a strong "Warburg effect", that is, they tend to obtain energy through high-rate glycolysis even in the presence of sufficient oxygen [17]. This process not only meets the bioenergy requirements of its rapid proliferation, but also generates a large amount of metabolites such as lactic acid in the local microenvironment, leading to extracellular space acidification and nu-

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Table 1. Distinctive biological features underlying the differential immunotherapy response between osteosarcoma and common epithelial cancers.

	Osteosarcoma	Common epithelial cancers (e.g., lung cancer, breast cancer)
Cellular & tissue origin	Arises from mesenchymal cells and directly produces osteoid matrix [2].	Originate from epithelial cells, forming glandular or squamous structures.
Core genomic features	Exhibits a highly complex and unstable genome without reliance on single driver mutations; characterized primarily by inactivation of tumor suppressor genes (e.g., *TP53*, *RB1*) and widespread chromosomal copy number variations [5].	Typically driven by specific, targetable oncogenic mutations [6,7] (e.g., *EGFR*, *ALK*, *HER2*).
Typical immune microenvironment	Often presents as an "immune-excluded" or "immune-desert" phenotype, where immune cells are either restricted to the stroma or severely lacking; marked by abnormal enrichment of immunosuppressive cells such as tumor-associated macrophages and regulatory T cells (Tregs) [8].	Highly heterogeneous; some subtypes (e.g., certain non-small cell lung cancers) can exhibit an "immune-inflamed" phenotype with significant tumor-infiltrating lymphocytes [9].
Metabolic reprogramming characteristics	Exhibits an extremely pronounced Warburg effect with highly active glycolysis and significant dependency on various nutrients like glutamine; metabolic plasticity is key for adapting to the microenvironment and facilitating metastasis [4,10].	Metabolic reprogramming exists but varies across cancer types; typically involves aerobic glycolysis, with its plasticity more closely linked to specific driver gene pathways.
Clinical & epidemiological features	Shows a classic bimodal age distribution (adolescents and adults >40 years); pulmonary metastasis is the primary cause of mortality [2].	Primarily occurs in middle-aged and elderly populations; metastatic sites are diverse (bone, brain, liver, lung, etc.).

TP53, Tumor Protein P53; RB1, Retinoblastoma Transcriptional Corepressor 1; EGFR, Epidermal Growth Factor Receptor; ALK, Anaplastic Lymphoma Kinase; HER2, Erb-B2 Receptor Tyrosine Kinase 2.

trient depletion [18,19]. This "acidic, hungry" metabolic environment deals a fatal blow to immune cells (such as effector T cells) that rely on specific metabolic programs to perform their functions, while facilitating the survival and activation of immunosuppressive cells [20].

Although immune checkpoint inhibitors such as Pembrolizumab and Nivolumab have shown some clinical activity in some patients with advanced osteosarcoma, the results of large clinical trials (such as the SARC028 study) suggest that the objective response rate was generally below 10–15% [21,22]. This suggests that only a small proportion of patients benefit from monotherapy, revealing the widespread primary drug resistance in osteosarcoma. The root cause of this resistance is largely attributed to the highly immunosuppressive tumor microenvironment (TME) of osteosarcoma. This microenvironment is not a mere "desertification" of immune cells, but a dysfunctional "ecosystem": Infiltrating cytotoxic CD8+ T cells often show functional "exhaustion", while immunosuppressive regulatory T cells (Tregs), M2-type macrophages, etc., are abnormally enriched, together forming a physical and biochemical barrier to protect the tumor [23,24].

In response to this challenge, the focus of research is shifting from a single immune blockade to how to effectively reshape the immune microenvironment. Targeting tumor metabolism has emerged as one of the most promising strategies. Osteosarcoma cells exhibit intense metabolic reprogramming (such as the Warburg effect), which not only directly inhibits the function of effector T cells by consuming a large amount of glucose and generating lactic acid, but also actively educates and recruits immunosuppressive cells to maintain immune escape [25]. Therefore, targeting these key metabolic pathways is seen as a key means to "disengage" microenvironment immunosuppression and transform "cold" tumors into "hot" ones. This review will systematically present the scientific basis and research progress of this new paradigm of "metabolic combined immunotherapy", and demonstrate its future direction as a way to overcome drug resistance in osteosarcoma treatment and improve patient prognosis.

2. Metabolic Reprogramming of Osteosarcoma and the Shaping of an "Acidic, Hungry" Microenvironment

Metabolic reprogramming is a key characteristic in the occurrence and development of osteosarcoma, profoundly influencing tumor progression, metastasis and drug resistance [26,27]. This process not only provides energy and biosynthetic precursors for the rapid proliferation of cancer cells themselves, but also creates an "acidic, hungry" local environment that is conducive to tumor survival while suppressing normal immune function by reshaping the TME.

2.1 The Warburg Effect and the Establishment of the Acidic Microenvironment

The Warburg effect, aerobic glycolysis, is the most prominent metabolic feature of osteosarcoma. Cancer cells take up large amounts of glucose and metabolize it into lactic acid, even in the presence of sufficient oxygen. This process is precisely regulated at multiple levels. *TRIM22*, for example, inhibits the Warburg effect with OS progression by activating ROS/AMPK/mTOR pathway-mediated autophagy [28]. Molecules such as *SPINT1-AS1*, *PDK1* and *HOTAIRM1* positively promote glycolysis by regulating glucose metabolism, activating the TGF-β/Smad pathway or the miR-664b-3p/Rheb/mTOR axis, and enhance the stemness and anti-apoptotic ability of tumor cells [29,30,31]. In addition, pyruvate kinase M2 (PKM2), as a key regulatory point of glycolysis, is regulated by molecules such as *lncCCAT1* and *IRF7*, further influencing the intensity of the Warburg effect and lipid synthesis [32,33].

The direct consequence of the Warburg effect is the massive accumulation of lactic acid in the TME, leading to persistent acidification of the microenvironment [34]. This acidic condition not only directly promotes tumor invasion and metastasis, but also inhibits the function of effector immune cells (such as CD8+ T cells), while inducing macrophages to polarize towards the tumor-promoting M2 phenotype [35], jointly constructing an immunosuppressive acidic TME (Fig. 1).

2.2 Abnormal Activation of Lipid and Amino Acid Metabolism and Nutritional Competition

In addition to glucose, osteosarcoma cells also show intense reprogramming in lipid and amino acid metabolism, exacerbating "nutritional hunger" in the TME.

2.2.1 Lipid Metabolism Remodeling

Lipids are an important component of biofilms and signaling molecules. Osteosarcoma cells enhance de novo lipid synthesis by upregulating key factors such as fatty acid synthase (*FASN*) and fatty acid binding protein 5 (*FABP5*) to meet the membrane structure required for their rapid proliferation [36]. circRNA (such as hsa_circ_0000073) and RNA modifications (such as NSUN2-mediated m5C modifications) are also involved, promoting lipid synthesis by regulating the expression of related genes [37]. Targeting aberrant lipid metabolic pathways—such as through the application of the traditional Chinese medicine monomer Brusatol or modulation of BRD4-mediated *ACSL3* splicing—has been demonstrated to effectively suppress osteosarcoma cell proliferation and induce ferroptosis, underscoring the significant therapeutic potential of this signaling axis [38,39].

2.2.2 Amino Acid Metabolic Dependence

Osteosarcoma cells show a high degree of dependence on specific amino acids, particularly glutamine and methionine. Glutamine is taken up in large quantities through the transporter *SLC38A5*, activating the PI3K/AKT/mTOR signaling pathway, promoting cell proliferation and inhibiting ferroptosis [40]. Likewise, macrophages respond to chemotherapy by modulating the *LAT2* transporter in tumor cells, enhancing leucine and glutamine uptake, and thereby promoting immune escape through the mTORC1/c-Myc axis [41]. In addition, methionine metabolism is crucial as its deficiency leads to impaired mitochondrial function [42], and intervention in methionine metabolism can also affect immunotherapy sensitivity by regulating *IKZF1* and influencing PD-L1 expression [42].

These metabolic activities make osteosarcoma cells a "nutrient black hole" in the TME, competing fiercely with infiltrating immune cells for key nutrient substrates such as glucose and glutamine, causing immune cells to dysfunction due to "starvation" and further strengthening immunosuppression.

2.3 Changes in Mitochondrial Function and Metabolic Adaptability

Metabolic reprogramming in osteosarcoma is also accompanied by profound changes in mitochondrial function. On the one hand, some osteosarcoma cells have reduced their dependence on oxidative phosphorylation, but mitochondria have not been inactivated; instead, their functions have been reconstructed to accommodate biosynthesis and survival requirements. Genes such as *LRPPRC*, *SLC25A10*, and *OMA1* are crucial for maintaining mitochondrial homeostasis and function, and their abnormal expression leads to mitochondrial dysfunction, REDOX imbalance, and ultimately affects tumorigenesis [43,44]. On the other hand, upregulation of mitochondrial protease YME1L is associated with metabolic activity and resistance to apoptosis in osteosarcoma [45].

This mitochondrial plasticity gives osteosarcoma cells the ability to cope with metabolic stress. For example, studies have shown that different cell lines have different dependencies on energy substrates: glycolysis-dependent cells are sensitive to glucose starvation, while glutamine-dependent cells are sensitive to glutamine deprivation [46]. This metabolic heterogeneity is also reflected in the stress response, where activation of the EGFR/PI3K/AKT pathway is crucial for cell survival under serum starvation conditions [47]. Autophagy-related gene *ATG4D* helps cells survive under starvation conditions by regulating autophagic flux [48].

2.4 Hypoxia: Drives Metabolic Reprogramming and Immunosuppression

Osteosarcoma typically exhibits hypoxic regions due to the rapid proliferation of tumor cells and abnormal vas-

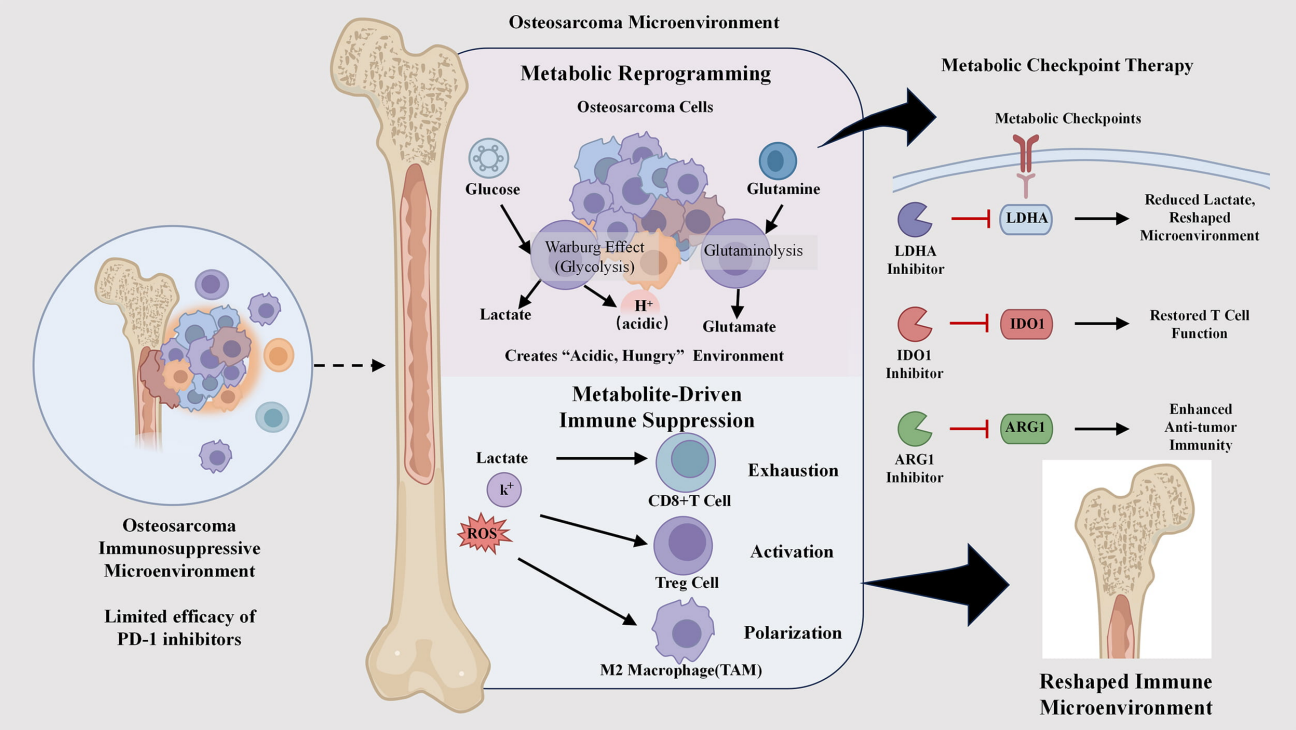


Fig. 1. Mechanisms of metabolic immunosuppression and therapeutic targeting in osteosarcoma. This illustration delineates the core mechanisms by which osteosarcoma cells promote tumor progression and shape an immunosuppressive tumor microenvironment (TME) through metabolic reprogramming, along with the corresponding intervention strategies. It presents the reprogramming of key metabolic pathways within osteosarcoma cells and summarizes potential therapeutic approaches targeting the aforementioned metabolism–immunity axis. TAMs, tumor-associated macrophages; LDHA, Lactate dehydrogenase A; IDO1, Indoleamine 2,3-dioxygenase 1; PD-1, Programmed Cell Death Protein 1; ROS, Reactive Oxygen Species; ARG1, Arginase 1. The figure was prepared using Microsoft PowerPoint (Version 2402, Microsoft Corporation, Redmond, WA, USA).

cular distribution [49]. Hypoxia is not merely a metabolic stress but also serves as a central driver that remodels tumor biological behavior, promotes immunosuppression, and confers therapy resistance through activation of the hypoxia-inducible factor (HIF) family [50].

When transcription factors such as HIF-1α are stably activated by hypoxia, they directly upregulate the expression of multiple key glycolytic genes including *GLUT1*, *LDHA*, and *PDK1* [50]. This not only intensifies the Warburg effect, leading to excessive lactate production and further acidification of the microenvironment [51], but also promotes angiogenesis (by upregulating *VEGF*) to meet the tumor's growing energy and nutrient demands. However, this newly formed vascular system is often dysfunctional, exacerbating the hypoxic vicious cycle [52].

The hypoxic microenvironment is a key factor in shaping the "cold tumor" immune phenotype. HIFs can upregulate the expression of immune checkpoint molecules such as PD-L1 on tumor cell surfaces, directly suppressing T-cell function [53]. Additionally, hypoxia can recruit and induce tumor-associated macrophages and myeloid-derived suppressor cells to differentiate into immunosuppressive phenotypes, thereby suppressing effective anti-tumor immune responses [54]. More importantly, hypoxia is one of the main factors leading to resistance to chemotherapy and radiotherapy. It can help tumor cells evade treatment by activating pro-survival signaling pathways such as NF-κB, enhancing DNA repair capacity, and inducing cell cycle arrest [51]. Nowadays, targeting the HIF pathway or exploiting the hypoxic microenvironment itself has become an emerging cancer treatment strategy.

3. Metabolic Reprogramming in Osteosarcoma and the Formation of an Immunosuppressive Microenvironment: The Core Role of Metabolites

The occurrence and development of osteosarcoma are closely related to the immunosuppressive properties of the TME, and the metabolic reprogramming of cancer cells is the core driving force shaping this immunosuppressive state. These metabolic changes not only meet the rapid proliferation requirements of tumor cells themselves, but also directly inhibit the function of key immune cells by releasing specific metabolites (such as lactic acid, reactive oxygen species, etc.), while promoting the activation and polarization of immunosuppressive cells (such as regulatory

T cells and M2-type macrophages), thereby forming a microenvironment conducive to tumor growth and metastasis [55].

3.1 Abnormal Glucose Metabolism and Suppression of Immune Cell Function

In osteosarcoma, abnormal glucose metabolism in cancer cells is exacerbated by multiple molecular mechanisms and directly leads to impaired immune cell function. The circular RNA CircECE1 inhibits the ubiquitination degradation of the c-Myc protein by binding to it, thereby suppressing the transcription of thioredoxin-interacting protein (*TXNIP*) and ultimately activating the Warburg effect to promote glucose metabolism and tumor progression [56]. Similarly, circCSPP1 has been reported to promote glucose uptake and proliferation in osteosarcoma cells by suppressing miR-200c maturation [57]. This metabolic reprogramming leads to the massive consumption of glucose in the TME by tumor cells, causing glucose starvation in immune cells (especially CD8+ T cells), thereby weakening their cytotoxic function. In addition, lactic acid, as the end product of glycolysis, accumulates in the TME and directly inhibits T cell activity and proliferation. Studies have shown that by combining anti-PD-L1 antibodies with nanoparticles containing CCL19 and oxaliplatin, PD-1/PD-L1 inhibition signals can be blocked, lactic acid-induced T cell failure can be alleviated, and their antitumor ability can be restored [58].

3.2 Lactic Acid Metabolism and the Activation of Regulatory T Cells (Tregs)

The accumulation of lactic acid not only inhibits effector T cells but also promotes the activation and function of immunosuppressive Treg cells. Lactic acid is bidirectionally transported between cells via monocarboxylic acid transporters (MCT1 and MCT4) and participates in glycolysis and oxidative respiration. Inhibition of MCT1 increases oxidative stress, reduces circulating tumor cells and metastasis, but MCT1 and MCT4 have functional redundancy in osteosarcoma, so combined inhibition of both may be an effective therapeutic strategy [59]. In osteosarcoma samples, Tregs are highly infiltrated, with metabolic characteristics such as oxidative phosphorylation, angiogenesis, and activation of the mTORC1 pathway. Tregs interact with osteoblasts, endothelial cells, etc. through CXCL signaling, further promoting tumor growth and metastasis. Prognostic models based on Tregs-related features showed good predictive value in multiple cohorts, and patients at high risk of Tregs were sensitive to specific targeted drugs [60]. This suggests that lactate metabolism plays a key role in immune escape in osteosarcoma by modulating Tregs function.

3.3 Metabolites and Polarization of M2-Type Macrophages

M2-type polarization of tumor-associated macrophages (TAMs) is another important feature of the immunosuppressive microenvironment of osteosarcoma [61]. Osteosarcoma cells induce macrophages to differentiate into M2 type through exosome-mediated Tim-3 signaling, which then secrete cytokines such as IL-10, TGF-β, and VEGF, promoting tumor cell migration, invasion, and lung metastasis [62]. Lactic acid and hypoxic environments further intensify this process. Studies have found that all-trans retinoic acid (ATRA) can block M2 polarization in TAMs by inhibiting IL-13-induced MMP12 secretion independently of STAT3/6 or C/EBPβ pathways, thereby reducing osteosarcoma lung metastasis [63]. In addition, methionine enkephalin (MENK) not only directly inhibits osteosarcoma cell proliferation by upregulating the opioid growth factor receptor (OGFr), but also reshapes the immune microenvironment by reducing the proportion of myeloid-derived suppressor cells (MDSCs), increasing the proportion of M1-type macrophages, and reducing the proportion of M2-type macrophages [64]. Another natural compound, Arjunolic acid (AA), reverses the malignant behavior of osteosarcoma cells by inhibiting Wnt3A-mediated macrophage M2 polarization [65]. These studies highlight the therapeutic potential of targeting metabolic pathways to regulate macrophage polarization.

In addition to soluble metabolites, tumor cell-derived exosomes are another class of key carriers that mediate intercellular communication and transmit immunosuppressive signals [52]. These nanoscale vesicles carry a variety of bioactive substances such as proteins, lipids, and nucleic acids (such as miRNAs and lncRNAs), which can systematically reshape the tumor microenvironment. Osteosarcoma cells by specific signals secrete body outside, active education immune cells in order to maintain the immune escape. For example, exosomes bearing Tim-3 protein can be taken up by macrophages to induce polarization toward a tumor-promoting M2 phenotype [66]. In addition, non-coding RNAs (such as lncRNAs and circRNAs) in exosomes can target metabolic-related genes in recipient cells and remotely regulate their metabolic program [67]. The cargo molecules of exosomes derived from hypoxic cells may be enriched in HIFs-regulated factors, thereby spreading the hypoxic signal to the further microenvironment and even to the premetastatic foci, creating favorable conditions for metastasis [68]. Therefore, targeting the biogenesis, secretion or uptake of tumor exosomes is expected to become an emerging strategy to interfere with the metabolism-immune dialogue and reverse immune suppression.

3.4 Comprehensive Treatment Strategies: Targeting the Intersection of Metabolism and Immunity

Comprehensive treatment strategies targeting the metabolic and immune cross-network of osteosarcoma have shown broad prospects. For example, CSF1/CSF1R signaling pathway inhibitor Pexidartinib (PLX3397) can reprogram TAMs, reduce M2-type polarization, while depleting TAMs and FOXP3+ Tregs and enhancing CD8+ T cell infiltration in primary and metastatic lesions [69]. This combined intervention not only alleviates the immunosuppression caused by the competition between lactic acid and glucose, but also restores the function of effector T cells.

4. Target Glucose Metabolism Checkpoints

4.1 LDHA and the Lactic Acid Microenvironment

Lactate dehydrogenase A (LDHA), one of the core enzymes in osteosarcoma glucose metabolism reprogramming, is responsible for converting pyruvate to lactic acid and is a key executor of the Warburg effect. LDHA is often significantly overexpressed in osteosarcoma cells, leading to a large amount of lactic acid production and acidification of the tumor microenvironment. Studies have shown that lactic acid is not only a metabolic waste but also an important signaling molecule that promotes immunosuppression through multiple mechanisms [70].

High circ-CNST/LDHA/PDK1 or low miR-578 expression may predict shorter overall survival, later TNM staging and lymph node metastasis in OS patients. Experiments showed that blocking circ-CNST and restoring miR-578 could enhance apoptosis rate, inhibit cell proliferation, clone formation, migration and invasion, and reduce glucose consumption, lactate production and ATP/ADP ratio [71]. KDM6B increases LDHA expression by directly mediating the demethylation of H3K27me3, and overexpression of LDHA can reverse the metastasis inhibition phenotype of KDM6B knockdown OS cells [72]. FX-11, as a specific LDHA inhibitor, significantly reduced lactate production in osteosarcoma transplanted tumor models and enhanced CD8+ T cell infiltration and function [73].

4.2 Other Key Targets in the Glycolytic Pathway

In addition to LDHA, there are other potential therapeutic targets in the osteosarcoma glycolytic pathway. Enolase 1 (ENO1) as a key enzyme in the glycolytic pathway plays a significant role in the metastasis of osteosarcoma. Studies using single-cell RNA sequencing technology found that ENO1 was significantly highly expressed in osteosarcoma samples with lung metastases [74]. The study suggests that ENO1 promotes migration and invasion of osteosarcoma cells by regulating metabolic reprogramming from glycolysis to oxidative phosphorylation.

Hexokinase 2 (HK2), as the rate-limiting enzyme of glycolysis, is also highly expressed in osteosarcoma. HK2 associates glycolysis with apoptotic signaling pathways by binding to the outer mitochondrial membrane, promoting the survival and proliferation of osteosarcoma cells [75]. In osteosarcoma cells, *HK2* knockdown reduced glucose consumption and lactate production, increased apoptosis, and impaired colony formation [76].

4.3 Target Amino Acid Metabolism Checkpoints

IDO1

Indoleamine 2, 3-dioxygenase 1 (IDO1), a key rate-limiting enzyme in the tryptophan-kynurenine metabolic pathway, is highly expressed in osteosarcoma and catalyzes the catabolism of tryptophan along the kynurenine pathway, leading to tryptophan depletion and accumulation of metabolites in the tumor microenvironment. Tryptophan deficiency can activate the GCN2 signaling pathway, causing T cell cycle arrest and functional inhibition. Metabolites such as kynurenine promote the differentiation and function of regulatory T cells through the aryl hydrocarbon receptor signaling pathway, further enhancing the immunosuppressive microenvironment [77].

In clinical practice, combinations of IDO1 inhibitors such as epacadostat with PD-1 inhibitors have been tested in multiple solid tumors [78]. *In vitro*, epacadostat inhibited IDO1 enzymatic activity in IFN-γ-stimulated MG63 and G292 osteosarcoma cells in a dose-dependent manner [79].

4.4 Target Other Metabolism-Related Checkpoints

4.4.1 Nucleotide Metabolism

Nucleotide metabolism plays a key role in chemotherapy resistance in osteosarcoma. Studies have shown that osteosarcoma cells enhance nucleotide synthesis through metabolic reprogramming to provide sufficient raw materials for DNA damage repair, thereby resisting the killing effect of DNA-targeted chemotherapy drugs such as cisplatin. The study revealed the core role of the RFWD3-PHGDH molecular axis in cisplatin resistance in osteosarcoma [80]. RFWD3, an E3 ubiquitin ligase, promotes PHGDH ubiquitination and proteasomal degradation, thereby conserving cellular NAD^+ and supporting de novo nucleotide biosynthesis and DNA repair in osteosarcoma.

4.4.2 Oxidative Stress Regulation

Reactive oxygen species play a dual role in the occurrence and development of osteosarcoma—moderate ROS promotes the proliferation of tumor cells and the activation of survival signaling pathways, while excessive ROS leads to cell damage and death [81]. Osteosarcoma cells maintain a dynamic balance of ROS by upregulating the antioxidant system, a process that is also closely associated with treatment resistance. A team study has revealed the core role of the NSUN6-m5C-PEXs axis in the regulation of oxidative stress and cisplatin resistance in osteosarcoma [82] (Table 2).

Table 2. Emerging therapeutic strategies targeting the metabolic-immunosuppressive axis in osteosarcoma.

	Target/Pathway	Representative agent/ Strategy	Primary mechanism of action	Research stage
Metabolic pathway inhibitors	LDHA (Lactate Dehydrogenase A)	FX-11	Inhibits LDHA, reduces lactate production, alleviates microenvironment acidosis, and enhances CD8+ T cell infiltration and function.	Preclinical
	HK2 (Hexokinase 2)	HK2 inhibitors	Inhibits the rate-limiting glycolytic enzyme HK2, disrupts cancer cell energy metabolism, and induces apoptosis.	Preclinical (Systemic toxicity noted as a limitation)
	IDO1 (Indoleamine 2,3-Dioxygenase 1)	Epacadostat, INCB024360	Inhibits IDO1 enzyme activity, counteracts tryptophan depletion and kynurenine accumulation, reverses Treg-mediated immunosuppression, and enhances T cell response.	Clinical Trials (Epacadostat + anti-PD-1 in other solids); Preclinical (INCB024360 in OS models)
Immune microenvironment modulators	CSF1R/Macrophages	Pexidartinib (PLX3397)	Inhibits CSF1/CSF1R signaling, reprograms tumor-associated macrophages (TAMs), reduces M2 polarization, depletes TAMs and Tregs, and enhances CD8+ T cell infiltration.	Clinical Trials
	Macrophage Polarization	All-Trans Retinoic Acid (ATRA)	Blocks IL-13-induced MMP12 secretion, inhibits M2 macrophage polarization, and reduces lung metastasis.	Preclinical
		Arjunolic Acid (AA)	Inhibits Wnt3A-mediated M2 macrophage polarization.	Preclinical
		Methionine Enkephalin (MENK)	Upregulates OGFr to directly inhibit tumor proliferation; reduces MDSCs, increases M1 while decreasing M2 macrophages, reshaping the immune landscape.	Preclinical
Novel delivery & combination strategies	Exosome/Drug Delivery	Doxorubicin-loaded engineered exosomes	Utilizes the natural targeting of exosomes for drug delivery, enhances tumor-specific accumulation, and reduces systemic toxicity (e.g., cardiotoxicity).	Preclinical
	Nanoparticle Systems	CCL19/Oxaliplatin nanoparticle + anti-PD-L1	Co-delivers cytokine and chemotherapy via nanoparticles, locally blocks PD-1/PD-L1 and alleviates lactate-induced T cell exhaustion, restoring T cell function.	Preclinical/Under Development
		Hydroxyapatite/ Bisphosphonate-based targeted nanocarriers	Enables bone-tumor-targeted drug delivery, increases local concentration, and minimizes systemic exposure and side effects.	Preclinical
Other targeted agents	Lipid Metabolism	Brusatol	Targets aberrant lipid metabolism, inhibits tumor cell proliferation, and induces ferroptosis.	Preclinical
	Glutamine Metabolism	Targeting SLC38A5 or the PI3K/AKT/mTOR pathway	Interferes with glutamine uptake or downstream signaling, inhibiting proliferation and potentially inducing ferroptosis.	Preclinical/Mechanistic Study

FX-11, a specific LDHA inhibitor; CSF1, Colony Stimulating Factor 1; CSF1R, Colony Stimulating Factor 1 Receptor; OGFr, opioid growth factor receptor; MMP12, Matrix Metalloproteinase 12; MDSCs, myeloid-derived suppressor cells; CCL19, C-C Motif Chemokine Ligand 19; PD-L1, Programmed Cell Death Ligand 1; PI3K, Phosphoinositide 3-Kinase; AKT, Protein Kinase B; mTOR, Mammalian Target of Rapamycin.

5. Conclusion

This review systematically presents an inseparable close link between the immunosuppressive microenvironment of osteosarcoma and the metabolic reprogramming of tumor cells. Osteosarcoma cells actively shape an "acidic, hungry, and metabolically waste-rich" immunosuppressive ecosystem through enhanced Warburg effect, amino acid deprivation, and lipid metabolism remodeling, which directly leads to functional exhaustion of CD8+ T cells and promotes the activation of immunosuppressive cells such as Treg and M2-type macrophages [62,63].

Metabolic reprogramming in osteosarcoma directly regulates immune cell function through metabolites such as lactic acid and ROS, forming an immunosuppressive microenvironment characterized by CD8+ T cell depletion, Treg activation, and M2-type macrophage polarization [60,64]. Future studies need to further clarify the role of other metabolites such as potassium ions and, more importantly, translating these findings into effective combination strategies represents a critical next step. The primary future opportunity lies in developing therapeutic approaches that simultaneously target metabolic pathways and immune checkpoints, thereby overcoming the current treatment bottleneck. This understanding takes us beyond the traditional view that metabolic reprogramming is merely a means of energy supply for tumor cells, and instead regards it as an interventionable core hub for synergistic therapy.

Metabolic pathways are shared by normal cells, particularly activated immune cells and rapidly proliferating cells. Potential on-target hematologic toxicity should be considered when targeting LDHA, as inducible deletion of *Ldha* in adult mice caused non-lethal hemolytic anemia [83]. For example, affecting both tumor and T cell metabolism at the same time can have a "kill a thousand enemies, lose eight hundred of your own" effect. Therefore, it is crucial to develop bone tumor-targeted delivery systems (such as hydroxyapatite or bisphosphonate-based nanocarriers) that can increase local drug concentrations in tumors while minimizing systemic exposure and toxic side effects.

Second, the pervasive metabolic heterogeneity of osteosarcoma demands a shift towards precision medicine approaches. Biomarker absence and patient stratification: Not all osteosarcoma patients have the same metabolic profile. At present, we lack reliable biomarkers to predict which patients will respond to specific metabolic interventions. Future research is in urgent need of integrating multi-omics data (metabolomics, transcriptomics, etc.) and using non-invasive imaging techniques (such as phosphorus magnetic resonance spectroscopy (^{31}P-MRS)) to identify osteosarcoma subtypes with specific metabolic dependencies for true precision medicine.

Spatiotemporal heterogeneity and dynamic monitoring: Metabolic heterogeneity exists in the primary and metastatic sites of osteosarcoma, and even in different regions within the same tumor. In addition, the metabolic state of the tumor is dynamically evolving during treatment. Thus, another key opportunity lies in developing and validating technologies (e.g., serial liquid biopsies for circulating metabolites or tumor cells, functional imaging) that can monitor metabolic adaptations in real-time. This capability will be essential for dynamically adjusting combination therapy regimens to prevent or overcome secondary drug resistance.

To effectively integrate these strategies, future research on metabolic combination immunotherapy for osteosarcoma must advance toward more precise, adaptive, and integrated clinical approaches. Success in this endeavor depends not only on the development of novel therapeutic agents but fundamentally on the parallel advancement of companion diagnostics and innovative clinical trial designs. Key components include: the rational design of sequential or intermittent treatment regimens—such as metabolic "pre-conditioning" to remodel the TME prior to immune checkpoint blockade; the use of biomaterials to establish localized, sustained-release drug depots for continuous modulation of the tumor microenvironment; and the implementation of adaptive, biomarker-driven clinical trial platforms—such as basket or umbrella trials—that enable efficient evaluation of multiple combination therapies and rapid identification of responsive patient subpopulations. Despite existing challenges, a deeper understanding of the intricate "metabolic crosstalk" between tumor and immune cells, combined with precisely timed and targeted interventions, holds promise for achieving transformative therapeutic breakthroughs in osteosarcoma—a disease long regarded as a stubborn clinical challenge—and ultimately improving patient outcomes.

Author Contributions

JL developed the idea, YL and NY wrote the manuscript, MIUK revised the paper. YL, NY, and MIUK made substantial contributions to the review through literature searching and screening, critical evaluation of relevant studies, organization and synthesis of research findings, and interpretation of the available evidence. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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