Harnessing multi-omics to revolutionize understanding and management of osteosarcoma: A pathway to precision medicine (Review)

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Abstract. Osteosarcoma, the most prevalent primary bone malignancy in children and adolescents, poses significant challenges due to its aggressive nature and propensity for metastasis. Despite advances in treatment, survival rates for high-risk patients remain unsatisfactory, underscoring the urgent need for innovative approaches. This review explores the vital role of multi-omics-integrating genomics, transcriptomics, proteomics and metabolomics-in unraveling the complex biological landscapes of osteosarcoma. By providing comprehensive insights into tumor heterogeneity, signaling pathways and metabolic reprogramming, multi-omics facilitates the identification of novel biomarkers and therapeutic targets. The objective of the present study was to highlight the transformative potential of multi-omics in enhancing the understanding and management of osteosarcoma, ultimately paving the way for personalized treatment strategies and improved patient outcomes. Through this synthesis, the study calls for a concerted effort to integrate multi-omics into clinical practice, fostering a more precise approach to osteosarcoma care.

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1. Introduction

Osteosarcoma is a highly aggressive primary bone malignancy characterized by the production of osteoid and bone tissue, predominantly affecting the metaphyseal regions of long bones, particularly the distal femur, proximal tibia and proximal humerus (1). It generally arises in adolescents and young adults, peaking between the ages of 10 and 20 years, during periods of rapid skeletal growth (1). Epidemiologically, osteosarcoma has an annual incidence of ~3-4 cases per million individuals in the general population, with a notable male predominance (~1.5:1) (2). Geographic variations in incidence have been observed, with certain studies indicating higher rates in African populations and lower frequencies in Asian cohorts (2). Recent advancements in genomic studies have identified possible genetic risk factors, including mutations in the tumor protein 53 (TP53) gene linked to Li-Fraumeni syndrome (3,4). Furthermore, previous exposure to radiation and certain chemotherapeutic agents have been implicated in the etiology of osteosarcoma. Understanding these epidemiological patterns and genetic predispositions is crucial for developing targeted screening and prevention strategies in at-risk populations (5).

The current standard of care for osteosarcoma primarily involves a multidisciplinary approach, combining neoadjuvant chemotherapy, surgical resection and adjuvant

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Abbreviations: TME, tumor microenvironment; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-inducible factor 1α; CXCR4, C-X-C chemokine receptor type 4; MMPs, matrix metalloproteinases; LDHA, lactate dehydrogenase A; YAP1, Yes-associated protein 1; mTOR, mammalian target of rapamycin; FGFR, fibroblast growth factor receptor; FN1, fibronectin 1; ICAM-1, intercellular adhesion molecule-1; BCAA, branched-chain amino acid; AKT1, AKT serine/threonine kinase 1; CLEC3A, C-type lectin domain family 3 member A; MAT1, ménage à trios 1; PD-1/PD-L1, programmed death-1/programmed death-ligand 1; CDK, cyclin-dependent kinase; EZH2, enhancer of zeste homologue 2; ctDNA, circulating tumor DNA; CMRG, copper metabolism-related gene

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chemotherapy (6). High-dose multi-agent chemotherapy, typically including doxorubicin, cisplatin and methotrexate, aims to shrink tumors before surgery and eliminate micrometastatic disease postoperatively (7). However, despite advances in treatment, outcomes remain suboptimal, with a 5-year survival rate of ~60-70% for localized disease and significantly lower for metastatic cases. Limitations in treatment efficacy arise from tumor heterogeneity, development of chemoresistance and late diagnosis (8). Furthermore, the potential for severe adverse effects from aggressive chemotherapy regimens poses significant challenges to patient quality of life (9). Emerging strategies that incorporate multi-omics analyses are being explored to identify novel therapeutic targets and improve individualized treatment plans.

Multi-omics is an integrative approach that combines various layers of biological information, such as genomics, transcriptomics, proteomics and metabolomics, to gain comprehensive insights into cancer biology (10). This concept facilitates the understanding of the complex molecular interplay driving tumorigenesis, progression and response to therapy (11). By simultaneously analyzing the genetic profiles (including gene mutations and epigenetic changes), gene (mRNA) expression, protein expression, other RNA expression and metabolic profiles of tumors, researchers can identify novel biomarkers and therapeutic targets that may be overlooked by traditional methods such as isolated genomic, transcriptomic or proteomic analyses (12). Recent studies have highlighted the potential of multi-omics to refine patient stratification and personalize treatment strategies, leading to improved outcomes in cancers like osteosarcoma (4,13). The advent of advanced technologies and data analytics further enhances the capacity to integrate these diverse data types, positioning multi-omics as a cornerstone of precision medicine in oncology (14).

The purpose of this review is to synthesize current advances in multi-omics technologies and their application in osteosarcoma research, highlighting how these integrative approaches can enhance the understanding of tumor biology, improve prognostic stratification and inform the development of personalized treatment strategies for achieving better patient outcomes.

2. The complexity of osteosarcoma

Genetic landscape of osteosarcoma. The genetic landscape of osteosarcoma is characterized by multiple recurrent mutations and dysregulated pathways that contribute to its pathogenesis (15). Key mutations commonly observed in osteosarcoma include alterations in the TP53, retinoblastoma protein 1 (RB1) and mouse double minute 2 (MDM2) genes (Fig. 1) (16-18). TP53, a well-established tumor suppressor gene, is frequently mutated in numerous cancers, including osteosarcoma, leading to the loss of its normal function in cell cycle regulation and apoptosis (19). Mutations in RB1, another critical tumor suppressor, disrupt the Rb-E2F pathway, promoting uncontrolled cellular proliferation and contributing to tumor growth (20). Meanwhile, MDM2 overexpression results in the degradation of p53, further exacerbating the dysregulation of apoptosis and cell survival (21).

These genetic alterations not only inform tumor behavior but also influence treatment response. For example, osteosarcoma tumors with TP53 mutations may exhibit resistance to conventional chemotherapy agents, necessitating alternative therapeutic approaches (22). Furthermore, specific molecular subtypes identified through genomic profiling can be targeted with novel therapeutic agents, emphasizing the importance of personalizing treatment based on the genetic characteristics of the tumor (23).

Role of the tumor microenvironment (TME). The TME plays a crucial role in shaping the behavior and progression of osteosarcoma (24). Comprising various cell types, including immune cells, fibroblasts, endothelial cells and extracellular matrix (ECM) components, the TME significantly influences tumor growth and metastasis (Fig. 1) (25). Interactions between osteosarcoma cells and immune cells, such as macrophages and T-cells, can modulate the immune response, either promoting tumor progression through immune evasion or enhancing anti-tumor activity (26).

Certain soluble factors within the TME can also affect the behavior of osteosarcoma cells (27). For instance, the release of cytokines and growth factors from stromal cells can promote angiogenesis and tumor-supportive inflammation, creating a favorable niche for tumor growth (28). Additionally, components of the ECM, such as collagen and fibronectin, can interact with integrins on the surface of osteosarcoma cells, triggering signaling pathways that enhance proliferation and migration (29). Understanding the complex interplay between osteosarcoma cells and their microenvironment holds potential for therapeutic intervention. Strategies aimed at modulating the TME, such as targeted therapies that disrupt tumor-stroma interactions or immune checkpoint inhibitors, are being explored in ongoing clinical trials (30).

Tumor heterogeneity. Tumor heterogeneity is a hallmark feature of osteosarcoma, characterized by variations in the cellular composition, genetic makeup and functional properties of the tumor (31). Histopathological analyses often reveal a mixture of osteoblastic, chondroblastic and fibroblastic cell types within osteosarcoma, reflecting the diverse lineage origins and differentiation states of the tumor (31).

This heterogeneity has profound implications for treatment resistance, as subpopulations of cells may exhibit differential responses to therapy. For instance, a subset of cancer stem-like cells has been identified in osteosarcoma that is thought to be responsible for tumor recurrence and metastasis due to their enhanced survival capabilities and resistance to conventional therapies (32). The presence of these resistant subpopulations can lead to incomplete responses to treatment, ultimately resulting in unfavorable prognostic outcomes (33).

Furthermore, the dynamic nature of tumor evolution complicates the understanding of treatment resistance (34). As tumors are exposed to therapeutic pressures, they may undergo clonal selection, leading to the emergence of resistant variants that contribute to disease treatment resistance progression (35). Integrating multi-omics approaches to profile the genetic, transcriptomic and proteomic landscapes of osteosarcoma will provide insights into the mechanisms of

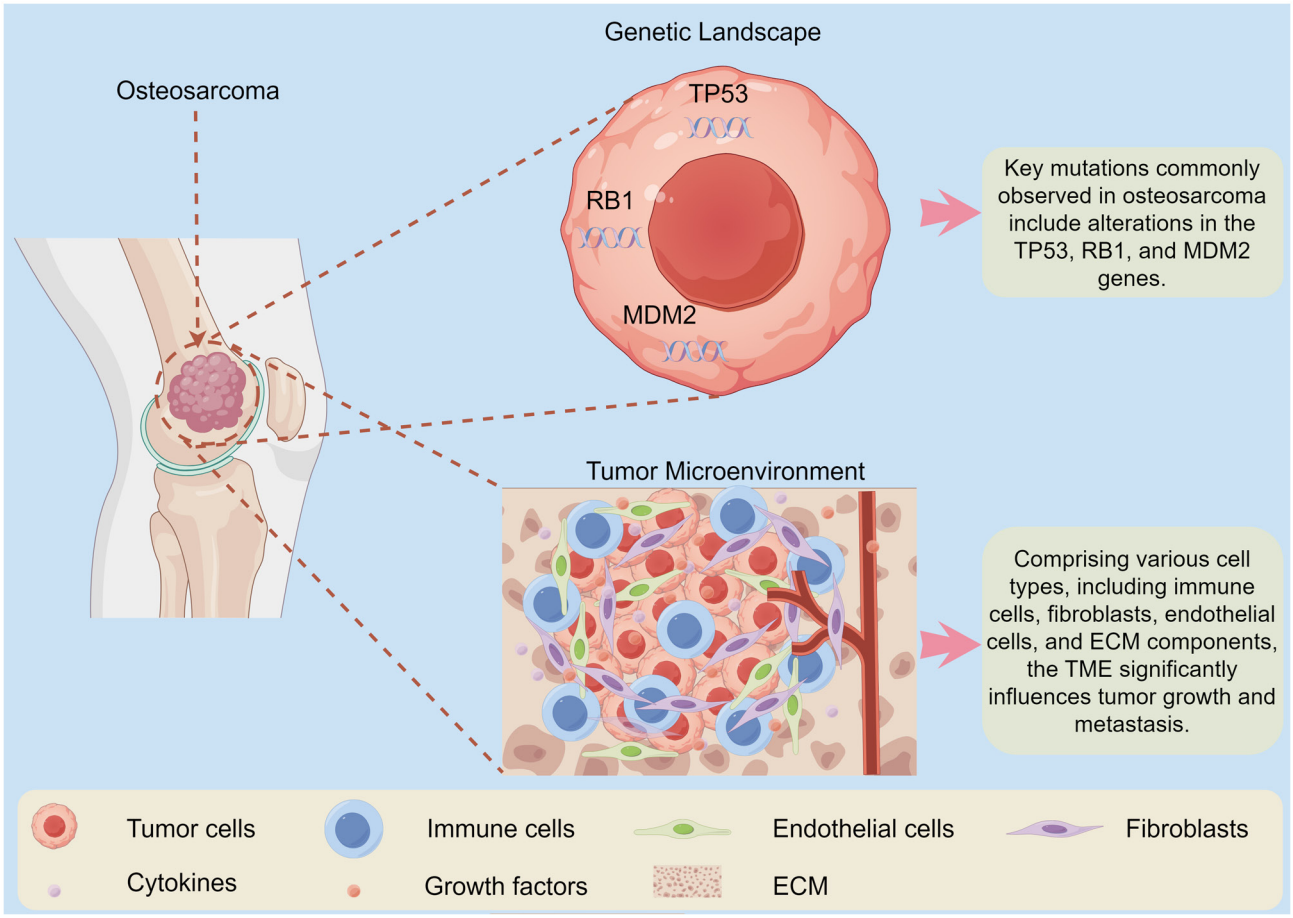

Figure 1. Schematic illustrating the characteristics of the genetic landscape and the TME of osteosarcoma. Osteosarcoma is characterized by a complex genetic landscape and TME. The figure shows key genetic alterations, including mutations in TP53, RB1 and MDM2, which contribute to osteosarcoma pathogenesis. In addition, it depicts the TME comprising immune cells, fibroblasts, endothelial cells and ECM components that influence tumor growth and metastasis. The interplay between genetic mutations and microenvironmental factors creates a conducive niche for tumor progression and therapeutic resistance. The figure was generated using Figdraw (https://www.figdraw.com). RB1, retinoblastoma protein 1; MDM2, mouse double minute 2; ECM, extracellular matrix; TME, tumor microenvironment; ECM, extracellular matrix.

heterogeneity and resistance, paving the way for the development of targeted therapeutic strategies tailored to specific tumor characteristics (36).

The complexity of osteosarcoma is a multifaceted challenge that necessitates a deeper understanding of its genetic landscape, the role of the TME and the heterogeneity within the tumor itself (37). Advances in multi-omics technologies hold promise for elucidating the underlying mechanisms driving osteosarcoma biology and can aid in the development of innovative therapeutic strategies that enhance patient outcomes (38). As we move towards precision medicine, a thorough comprehension of these complexities will be indispensable in addressing the limitations of current treatment paradigms.

3. Overview of multi-omics approaches

Genomics. Recent advances in whole-genome sequencing (WGS) have significantly enhanced our understanding of the genomic landscape of osteosarcoma (39). WGS allows for the identification of genetic alterations, comprehensively analyzing both coding and non-coding regions of the genome (40). Studies have identified critical driver mutations in key genes such as TP53 (16), RB1 (41) and MDM2 (42), which play pivotal roles in tumor development and progression (Table I).

Mutations in key tumor suppressor genes. Mutations in the TP53 gene are frequently observed in osteosarcoma, which are critical for tumor progression (22). These mutations lead to the inactivation of its tumor suppressor function, compromising the cellular mechanisms of apoptosis and DNA repair (22). A comprehensive study has established that germline TP53 mutations significantly increase susceptibility to osteosarcoma and correlate with poorer survival outcomes (16). The RB1 is another crucial tumor suppressor associated with osteosarcoma. Deletions or mutations in RB1, frequently observed in conjunction with TP53 mutations, enhance cell cycle progression and contribute to oncogenesis (41). It is noteworthy that the co-mutation of TP53 and RB1 is often linked to aggressive disease phenotypes and metastasis (17).

Amplification of oncogenes. Amplification of the MDM2 gene, which encodes a protein that inhibits TP53, is present in ~10-15% of osteosarcoma cases. Increased MDM2 expression facilitates the bypass of TP53-mediated apoptosis, aiding in tumor growth and resistance to therapies (42-44). Its role in the differentiation of osteosarcoma from benign lesions highlights its diagnostic significance (45). The PI3K/AKT

Table I. Genomics findings in osteosarcoma research.

Author/s, year	Potential targets	Samples	Major findings	(Refs.)
Mirabello *et al*, 2015	TP53	765 osteosarcoma cases	The genetic susceptibility of osteosarcoma with early onset differs from that of osteosarcoma with late onset, characterized by a high frequency of exon mutations in the TP53 gene.	(16)
Li *et al*, 2022	RB1	Mouse model	RB1 functions as a tumor suppressor in osteosarcoma by regulating glucose metabolism through the inhibition of YAP, with its loss leading to increased expression of Glut1 and enhanced tumor progression.	(41)
Mokánszki *et al*, 2020	RB1	A patient with retinoblastoma	RB1 functions as a tumor suppressor in osteosarcoma by regulating cell cycle progression and preventing tumorigenesis, with mutations leading to tumor development in patients with retinoblastoma.	(17)
Feng *et al*, 2020	MDM2	596 patients with osteosarcoma and 1,696 healthy controls	MDM2 acts as an oncogene in osteosarcoma by inhibiting the tumor suppressor function of p53, leading to enhanced tumor progression and poor patient outcomes.	(42)
He *et al*, 2018	MDM2	22 patients with low-grade osteosarcoma	MDM2 functions as an oncogene in low-grade osteosarcoma by promoting cellular proliferation through amplification, thereby contributing to tumorigenesis.	(43)
Limbach *et al*, 2020	MDM2	4 cases of primary osteosarcoma	MDM2 acts as an oncogene in craniofacial osteosarcoma by facilitating tumor differentiation and progression through gene amplification, aiding in distinguishing malignant tumors from benign lesions.	(44)
Kaur *et al*, 2022	MDM2	101 tissue samples diagnosed as osteosarcoma, ossifying fibroma, fibrous dysplasia or fibrous hyperplasia	MDM2 functions as an oncogene in osteosarcoma by promoting cell proliferation through gene amplification, aiding in differentiation from benign lesions.	(45)
Ren *et al*, 2020	CLEC3A	Tissue samples from patients with osteosarcoma from Gene Expression Omnibus dataset GSE99671	CLEC3A functions as an oncogene in osteosarcoma by promoting cell proliferation and reducing chemosensitivity through the AKT1/mTOR/HIF1α signaling pathway.	(46)
Wang *et al*, 2019	MAT1	Osteosarcoma tissue samples with and without lung metastasis	MAT1 promotes lung metastasis in osteosarcoma by enhancing cell proliferation, migration and invasion through increased AKT1 expression, correlating with poor patient prognosis.	(47)
Huang *et al*, 2023	FGF2	Human osteosarcoma tissue sections	FGF2 promotes osteosarcoma metastasis by enhancing cell migration and invasion through the activation of FGFR signaling and up-regulation of ICAM-1 expression.	(49)
Kim *et al*, 2022	FGFR1	Osteosarcoma tissues	FGFR1 facilitates radiation resistance in osteosarcoma by promoting cell survival and polyploidy through nuclear localization and activation of specific signaling pathways.	(50)

Table I. Continued.

Author/s, year	Potential targets	Samples	Major findings	(Refs.)
Makise *et al*, 2018	H3K27me3	19 cases of extraskeletal osteosarcoma	H3K27me3 functions as a transcriptional repressor in osteosarcoma, with its deficiency correlating with aggressive disease and poor patient outcomes.	(52)
Saba *et al*, 2019	FN1-FGFR1	A single case of chondroblastoma-like osteosarcoma	The FN1-FGFR1 gene fusion promotes oncogenic signaling in chondroblastoma-like osteosarcoma, potentially contributing to its distinct genetic profile and malignant behaviors.	(53)

RB1, retinoblastoma protein 1; YAP, Yes-associated protein; Glut1, glucose transporter 1; MDM2, mouse double minute 2; CLEC3A, C-type lectin domain family 3 member A; MAT1, ménage à trios 1; AKT1, thymoma viral proto-oncogene 1; FGF2, fibroblast growth factor 2; FGFR, FGFr receptor; ICAM-1, intercellular adhesion molecule; FN1, fibronectin 1.

signaling pathway is frequently activated in osteosarcoma. For instance, a study on C-type lectin domain family 3 member A demonstrated that its suppression led to reduced cell proliferation through the AKT serine/threonine kinase 1 (AKT1)/mammalian target of rapamycin (mTOR) axis (46). Furthermore, ménage à trios 1 has been shown to facilitate lung metastasis via upregulation of AKT1 expression, underscoring the pathway's role in metastatic spread (47).

Alterations in growth factor receptors. The fibroblast growth factor receptor (FGFR) signaling pathway has gained attention due to its involvement in osteosarcoma progression (48). The FGFR signaling pathway, particularly FGFR1, plays a critical role in the progression of osteosarcoma. Overexpression of FGF2 and FGFRs has been linked to increased cell migration, invasion and metastasis, notably through intercellular adhesion molecule-1 expression (49). Furthermore, nuclear FGFR1 contributes to radiation resistance by inducing cell survival mechanisms, such as G-2 checkpoint adaptation and histone modifications. Targeting FGFR signaling may not only hinder tumor progression but also enhance the efficacy of radiation therapy in osteosarcoma, providing a promising therapeutic strategy (50).

Epigenetic modifications. Aberrant epigenetic modifications have been identified in osteosarcoma that may contribute to its aggressiveness (51). For instance, alterations in histone markers, such as H3K27me3, have been noted in various osteosarcoma subtypes, indicating heterogeneous disease behavior (52). Such findings emphasize the need for integrating epigenetic profiling into the genomic landscape of osteosarcoma. Recent genomic profiling has identified fusion genes, such as fibronectin 1-FGFR1, which present new therapeutic targets (53). Such fusions indicate a need for targeted sequencing in osteosarcoma patients, particularly those with refractory disease, as they may provide insights into novel treatment strategies.

The integration of genomic findings into clinical practice presents an opportunity for personalized medicine in osteosarcoma. Genetic profiling can direct the selection of targeted therapies, optimize chemotherapeutic regimens and enhance the monitoring of disease progression. For instance, the detection of MDM2 overexpression may prompt the use of specific MDM2 inhibitors, tailoring treatment to the genetic landscape of the tumor (43,45).

The totality of genomic research on osteosarcoma illustrates a landscape of intricate genetic alterations that drive tumorigenesis (54). The mutations in tumor suppressor genes like TP53 and RB1, the amplification of oncogenes such as MDM2 and AKT1, and disturbances to growth factor signaling pathways underscore the heterogeneous nature of this malignancy (16,41,42,47). Advancements in understanding these changes are crucial not only in elucidating osteosarcoma biology but also in providing a framework for developing targeted therapies and precision medicine approaches. Future research should focus on synthesizing these genomic insights with clinical outcomes to enhance treatment efficacy and patient survival rates.

Transcriptomics. The application of transcriptomics in the study of osteosarcoma has unveiled significant insights into the molecular mechanisms underlying this aggressive bone tumor (55). By analyzing various RNA types, particularly

messenger RNA, researchers have identified crucial genes and pathways involved in osteosarcoma tumorigenesis, metastasis and response to therapy (Table II). This summary highlights key findings related to specific gene features, emphasizing their implications for precision medicine in osteosarcoma management.

Oncogenes and tumor suppressor genes. Mutations in the TP53 gene are prevalent in numerous cancers, including osteosarcoma (56). Research indicates that TP53 missense mutations are associated with longer survival in a canine model of osteosarcoma, suggesting a potential survival advantage linked to specific mutations (57). Furthermore, the impairment of rigid sensing due to mutant TP53 gain-of-function mutations has been noted, implicating TP53 in the modulation of cellular mechanics and tumor progression (58). The myelocytomatosis viral oncogene homolog (Myc) oncogene is pivotal in osteosarcoma pathogenesis. A study demonstrated that Myc not only acts as a poor prognostic biomarker but also serves as a potential therapeutic target, highlighting its critical role in the proliferation of osteosarcoma cells (59). Furthermore, the promotion of osteosarcoma development through WW domain containing oxidoreductase-mediated upregulation of Myc adds another layer of complexity to Myc's role in this disease (60). The downregulation of GATA binding protein 3 has been linked to the epithelial-to-mesenchymal transition (EMT) and migration of osteosarcoma cells. Its regulatory effects on Slug indicate its significance in managing metastatic potential in osteosarcoma (61).

Pathways and molecular mechanisms. Vascular endothelial growth factor (VEGF)/VEGF receptors: VEGF is essential for angiogenesis and has been shown to be associated with poor prognosis in patients with osteosarcoma (62). The dual inhibition of VEGF and survivin has exhibited proliferation inhibition and induced apoptosis in osteosarcoma cells (63), thereby presenting a promising therapeutic strategy. In addition, the splicing factor YBX1 promotes osteosarcoma progression by upregulating VEGF and downregulating its anti-angiogenic isoform VEGF (64). Hypoxia-inducible factor 1α (HIF-1α) mediates several processes involved in osteosarcoma, including cellular response to hypoxia and metabolism. Of note, HIF-1α-mediated augmentation of certain microRNAs facilitates proliferation and metastasis, reflecting its role in EMT pathways (65). Furthermore, the interplay between HIF-1α and mTOR signaling pathways is critical, as demonstrated by the synergistic anti-tumor activity of ginsenoside Rg3 alongside doxorubicin (66).

Cell migration and invasion. The C-X-C motif chemokine receptor 4 (CXCR4) is crucial for osteosarcoma cell migration and metastasis (67). Studies have shown that CXCR4 interacts with mesenchymal stem cells to promote tumor growth and pulmonary metastasis, primarily through VEGF signaling (68). Additionally, the CXCR4-coactivator associated arginine methyltransferase 1 (CARM1)-Yes-associated protein 1 (YAP) axis has been implicated in overcoming doxorubicin resistance in osteosarcoma by suppressing aerobic glycolysis, underscoring potential therapeutic pathways targeting these interactions (69). Expression of matrix metalloproteinase (MMP)-2 and MMP-9 is associated with poor prognosis and higher metastatic potential in osteosarcoma (70). Furthermore, the macrophage recruitment factor monocyte chemoattractant protein-1 enhances cancer cell migration through the c-Raf/MAPK/AP-1 pathway, further indicating the role of MMPs in the metastatic cascade (71).

Therapeutic implications and precision medicine. The integration of transcriptomic data in understanding the biology of osteosarcoma heralds a shift towards precision medicine approaches (72). Identifying specific gene expression patterns and their associated pathways allows clinicians to tailor treatment strategies based on individual molecular profiles, potentially improving outcomes. For example, targeting pathways involving TP53, HIF-1α and Myc provides a framework for developing targeted therapies that address the unique characteristics of each patient's tumor (59,73,74). Furthermore, the combination of transcriptomic and other omics data (e.g., proteomics, metabolomics) is expected to revolutionize the understanding and management of osteosarcoma. It can enable the identification of novel biomarkers for early diagnosis, prognosis and the development of innovative therapeutic strategies (75).

The application of transcriptomics in osteosarcoma research has significantly contributed to unravelling the complexities of this malignancy. Key findings regarding oncogenes, tumor suppressor genes, signaling pathways and non-coding RNAs provide a rich resource for future therapeutic strategies (76). The path toward precision medicine in osteosarcoma is being paved by these insights, which have the potential to enhance patient outcomes and transform clinical practices in oncology.

Proteomics. Recent advances in proteomics have illuminated the complexity of its pathology, leading to insights into specific proteins that drive cell proliferation, survival, invasion and metastasis (77). In the chapter below, key findings from proteomic studies in osteosarcoma are being discussed, focusing on significant protein classes such as cyclins, signaling proteins, apoptosis-related proteins, invasion/metastasis proteins, metabolic proteins, transcription factors and other targeted proteins (Table III).

Role of TME-related proteins. The TME plays a crucial role in osteosarcoma progression and metastasis, largely driven by signaling pathways mediated by proteins such as RAGE, HIF-1α and CXCR4 (78). Chang *et al* (79) elucidate that Nε-(1-carboxymethyl)-L-lysine activates RAGE signaling, driving osteosarcoma cell metastasis via ERK/NFκB axis. This suggests a significant link between advanced glycation end products and sarcomagenesis. Overexpression of CXCR4 has been tightly correlated with disease progression and poor prognosis in osteosarcoma. Specifically, CXCR4 signaling has been shown to facilitate myeloid-derived suppressor cell accumulation, which can blunt responses to immunotherapy (80). Furthermore, CXCR4 blockade has been proven to sensitize osteosarcoma cells to doxorubicin through autophagic cell death induction and inhibition of the PI3K-Akt-mTOR pathway (81). HIF-1α, a key regulator in the tumor response to hypoxic conditions, enhances osteosarcoma proliferation and metastasis through various proteins, such as matrilin 4 (MATN4). HIF-1α mediates the expression of MATN4, promoting progression (82), indicating the dual role of hypoxic signaling in metabolic reprogramming and tumor aggressiveness.

Table II. Transcriptomics findings in osteosarcoma research.

Author/s, year	Potential targets	Samples	Major findings	(Refs.)
Das *et al*, 2021	TP53	26 samples of osteosarcoma from dogs	TP53 functions as a critical tumor suppressor in osteosarcoma by regulating cell cycle progression and apoptosis, with its mutations linked to poorer outcomes.	(57)
Luo *et al*, 2023	TP53	Several osteosarcoma cell lines	TP53 functions as a tumor suppressor in osteosarcoma and its R156P mutation promotes tumorigenicity by inhibiting rigidity sensing, facilitating anoikis resistance.	(58)
Feng *et al*, 2020	Myc	A tissue microarray constructed from 70 patient samples	Myc functions as an oncogene in osteosarcoma, promoting tumor growth and metastasis, with its overexpression linked to poor patient prognosis.	(59)
Akkawi *et al*, 2024	Myc	A traceable osteosarcoma mouse model	Myc acts as an oncogenic driver in osteosarcoma, promoting tumorigenesis through upregulation of its target genes, particularly in the context of WWOX and TP53 deletion.	(60)
Ma *et al*, 2018	GATA3	GATA3 expression in osteosarcoma cells and tissues	GATA3 functions as a tumor suppressor in osteosarcoma by inhibiting cell proliferation, migration and invasion through the transcriptional regulation of the EMT-associated factor slug.	(61)
Wu *et al*, 2019	VEGF	Osteosarcoma samples from a cohort of 53 patients	VEGF promotes angiogenesis in osteosarcoma, and its high expression is associated with aggressive tumor behavior and poorer patient prognosis.	(62)
Gu *et al*, 2019	VEGF	Two human osteosarcoma cell lines	VEGF facilitates angiogenesis in osteosarcoma, and its inhibition can significantly reduce tumor proliferation and invasion.	(63)
Quan *et al*, 2023	VEGF	18 patients with osteosarcoma	VEGF promotes osteosarcoma progression by enhancing cell proliferation, migration, invasion and angiogenesis, while its isoform VEGF exerts antitumor effects and is downregulated in the disease.	(64)
Luo *et al*, 2022	HIF-1α	Human osteosarcoma tissues and cell lines	HIF-1α promotes osteosarcoma development by inducing the expression of miR-18b-5p, which inhibits the tumor suppressor PHF2, thereby enhancing cell proliferation and metastasis.	(65)
Zeng *et al*, 2023	HIF-1α	Human osteosarcoma cell lines	HIF-1α contributes to osteosarcoma progression by regulating the expression of key angiogenic and metastatic factors, including VEGF, through the mTOR signaling pathway.	(66)
Zhang *et al*, 2013	CXCR4	Human osteosarcoma cells	CXCR4 facilitates osteosarcoma growth and pulmonary metastasis by mediating the interaction between tumor cells and MSCs, enhancing VEGF secretion and promoting tumorigenic processes.	(68)

Table II. Continued.

Author/s, year	Potential targets	Samples	Major findings	(Refs.)
Li *et al*, 2024	CXCR4	Doxorubicin-resistant osteosarcoma cell lines	CXCR4 contributes to osteosarcoma doxorubicin resistance by regulating the CARM1-YAP signaling axis, which controls aerobic glycolysis and affects tumor cell survival and metabolism.	(69)
Gong *et al*, 2020	CXCR4	Pathological samples of 73 patients with osteosarcoma	CXCR4 facilitates the invasion and metastasis of osteosarcoma by correlating with MMP-2 expression, serving as a significant prognostic indicator for patient outcomes.	(70)
Liu *et al*, 2020	MMP9	Osteosarcoma cell lines	MMP-9 facilitates osteosarcoma progression and metastasis by mediating cell migration and invasion pathways regulated by MCP-1 signaling.	(71)

Myc, myelocytomatosis viral oncogene homolog; WWOX, WW domain-containing oxidoreductase; GATA3, GATA binding protein 3; EMT, epithelial-mesenchymal transition; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-Inducible factor 1α; PHF2, PHD finger protein 2; mTOR, mammalian target of rapamycin; CXCR4, C-X-C chemokine receptor type 4; CARM1, coactivator associated arginine methyltransferase 1; YAP, yes-associated protein 1; MSCs, mesenchymal stem cells; MMP9, matrix metallopeptidase 9; MCP-1, monocyte chemoattractant protein-1.

Metastasis-related proteins. Metastasis is a complex biological process that involves multiple signaling pathways, with several proteins acting as key facilitators. MMPs, particularly MMP-2 and MMP-9, are crucial for ECM degradation, contributing to metastasis (83). Overexpression of mucin (MUC)15 has been associated with enhanced osteosarcoma cell proliferation and invasiveness via the Wnt/β-catenin signaling pathway and regulated through MMP-2 and MMP-9 (74). A study indicated that sirtuin 6 negatively regulates proliferation and invasion in osteosarcoma by targeting N-cadherin (84), showcasing how proteins involved in EMT are leveraged by osteosarcoma cells to facilitate metastatic spread.

Apoptosis and survival pathways. Proteomic studies have illuminated various pathways by which osteosarcoma cells evade programmed cell death, revealing potential targets for apoptosis-inducing therapies (77). Proteins such as survivin and Livin have been highlighted as important mediators of apoptosis resistance in osteosarcoma. MUC15 was shown to promote cell proliferation through the Livin protein, linking the Wnt/β-catenin pathway with survival (74). The B-cell lymphoma-2 (Bcl-2) inhibitor AT-101 has demonstrated efficacy in inhibiting osteosarcoma growth in preclinical models (85). Further exploration of how survival pathways modulated by Bcl-2 family members interact with proteomics will enhance our understanding of therapeutic resistance.

Metabolic regulation of osteosarcoma. The metabolic profile of osteosarcoma cells is essential for their proliferation and survival. Critical proteins involved in glycolysis and oxidative phosphorylation are frequent subjects of investigation (86). A study indicated that lysine-specific demethylase 6B-mediated histone demethylation of lactate dehydrogenase A (LDHA) promotes lung metastasis in osteosarcoma. This highlights the role of LDHA in shaping the metabolic landscape (87). Furthermore, N-acetyltransferase 10-mediated ac4C acetylation of LDHA was shown to upregulate glycolytic metabolism in osteosarcoma (88). Aerobic glycolysis: The CXCR4-CARM1-YAP axis has been found to be critical in regulating glucose metabolism, linking aerobic glycolysis to chemoresistance (69). Targeting these metabolic pathways may thus provide another avenue for intervention.

Potential therapeutic targets. Overall, the insights gleaned from proteomics research reveal numerous potential therapeutic targets for osteosarcoma. Inhibition of motif-containing family genes 26 (TRIM26) offers a potential therapeutic strategy by destabilizing RACK1 and inactivating MEK/ERK signaling, demonstrating its role in osteosarcoma progression (89). Evidence suggests that Zyxin and TRIM22 restrict proliferation and metastasis via diverse signaling pathways, providing promising avenues for therapeutic explorations (90,91). The splicing factor Y-box binding protein 1 (YBX1) has been identified as a promoter of osteosarcoma progression through the upregulation of VEGF (64). Intervening in YBX1 pathways may effectively inhibit angiogenesis and tumor growth.

The ongoing proteomics research into osteosarcoma extends our understanding of tumor biology, highlighting crucial proteins that underlie molecular pathways involved in tumor progression, metastasis and survival (92). These proteins offer promising targets for new therapeutic strategies, directing future research toward enhancing targeted therapies and personalized medicine approaches for osteosarcoma. As

Table III. Proteomics findings in osteosarcoma research.

Author/s, year	Potential targets	Samples	Major findings	(Refs.)
Chang *et al*, 2024	CML	Human osteosarcoma tissues	CML promotes osteosarcoma progression by enhancing cell migration, invasion and stemness through the activation of the RAGE receptor and downstream ERK/NF-κB signaling pathways.	(79)
Jiang *et al*, 2019	CXCR4	Osteosarcoma tissues and murine models	CXCR4 facilitates immune evasion in osteosarcoma by promoting the migration of MDSCs, which inhibit cytotoxic T-cell expansion and reduce their apoptosis through SDF-1 signaling.	(80)
Liao *et al*, 2021	CXCR4	Osteosarcoma cell lines	CXCR4 facilitates osteosarcoma chemoresistance by regulating autophagy processes, with its inhibition enhancing doxorubicin-induced apoptosis and sensitizing osteosarcoma cells to chemotherapy.	(81)
Zhang *et al*, 2024	MATN4	Osteosarcoma tissues and cell lines	MATN4 promotes osteosarcoma progression by enhancing cell proliferation, migration and invasion under hypoxic conditions, regulated by HIF-1α.	(82)
Gao *et al*, 2019	SIRT6	Osteosarcoma tissues and cell lines	SIRT6 functions as a tumor suppressor in osteosarcoma by inhibiting cell proliferation and invasion through the downregulation of N-cadherin.	(84)
Masuelli *et al*, 2020	Bcl-2	Human and murine osteosarcoma cells	Bcl-2 promotes osteosarcoma cell survival and resistance to therapy by inhibiting apoptosis, making it a critical target for therapeutic interventions.	(85)
Jiang *et al*, 2021	LDHA	Osteosarcoma specimens	LDHA promotes osteosarcoma metastasis by enhancing glycolysis and facilitating tumor cell migration, regulated by the demethylation activity of KDM6B.	(87)
Mei *et al*, 2024	NAT10	Human osteosarcoma tissues	NAT10 promotes osteosarcoma progression by regulating m(6)A modification and glycolysis through the stabilization of mRNA for key glycolytic enzymes, mediated by YTHDC1.	(88)
Xia *et al*, 2023	TRIM26	Osteosarcoma tissues and cell lines	TRIM26 inhibits osteosarcoma progression by promoting the degradation of RACK1, leading to the inactivation of MEK/ERK signaling and suppression of EMT.	(89)

CML, Nε-(1-carboxymethyl)-L-lysine; RAGE, receptor for advanced glycation end products; ERK, extracellular signal-regulated kinase; CXCR4, C-X-C chemokine receptor type 4; MDSCs, myeloid-derived suppressor cells; SDF-1, stromal cell-derived factor 1; MATN4, matrilin-4; HIF-1α, hypoxia-inducible factor 1α; SIRT6, silent mating type information regulation 2 homolog 6; Bcl-2, B-cell lymphoma-2; LDHA, lactate dehydrogenase A; KDM6B, lysine-specific demethylase 6B; NAT10, N-acetyltransferase 10; YTHDC1, YTH N6-methyladenosine RNA binding protein C1; TRIM26, motif-containing family genes 26; RACK1, receptor for activated C kinase 1; MEK, mitogen-activated protein kinase kinase; EMT, epithelial-to-mesenchymal transition.

proteomic data are being harnessed, integrating these insights with other omics layers will be pivotal in advancing precision medicine for osteosarcoma management.

Metabolomics. The exploration of metabolomic changes in osteosarcoma has opened up several metabolic pathways that are responsible for the disease's progression and therapeutic resistance (93). The summary provided in the chapter below delineates findings from recent studies, emphasizing various metabolic pathways related to osteosarcoma, with particular attention paid to lipid and glucose metabolism, amino acid metabolism and the role of TME interactions (Table IV).

Lipid metabolism. Lipid metabolism has emerged as a critical player in osteosarcoma pathophysiology. Research indicates that deregulated lipid homeostasis is linked to cancer progression and metastasis in osteosarcoma (94). For instance, Hu *et al* (95) demonstrated that sphingolipid metabolism is intricately associated with osteosarcoma metastasis and patient prognosis. Furthermore, studies suggest that long non-coding RNAs, such as RPARP-antisense RNA 1, can regulate lipid metabolism, thus promoting osteosarcoma progression (96). Additionally, Bispo *et al* (97) have shown that novel metal-based drugs can impact lipid metabolism in MG-63 osteosarcoma cells, presenting a potential therapeutic avenue. Notably, fatty acid oxidation and the associated signaling pathways are essential for the survival and growth of osteosarcoma cells. Research by Fritsche-Guenther *et al* (99) highlighted the shifting nutrient dependencies of osteosarcoma cells, revealing that certain metabolic adaptations facilitate their aggressive traits. The interplay between lipid droplets and the osteosarcoma microenvironment is thus critical for understanding the tumor's metabolism.

Glucose metabolism. The Warburg effect, characterized by increased glycolysis in the presence of oxygen, is prevalent among osteosarcoma cells. Shen *et al* (99) noted that circular RNAs, such as Hsa_circ_0000566, promote glycolysis and contribute to osteosarcoma progression by feedback regulation on HIF-1α. Similarly, Li *et al* (100) indicated that aberrations in glucose metabolism, specifically through the upregulation of LDHA by miR-329-3p, sensitize osteosarcoma cells to cisplatin treatment, underscoring the relevance of metabolic pathways for therapeutic responses. Furthermore, elevated glucose levels have been correlated with enhanced stemness and metastatic potential in osteosarcoma. Wang *et al* (101) demonstrated that modulating glucose metabolism affects the cancer stem cells' properties and their tumorigenicity. Targeting these metabolic pathways provides opportunities to enhance the efficacy of existing therapies.

Amino acid metabolism. Amino acid metabolism, particularly glutamine metabolism, has gained attention in cancer research due to its association with tumor growth and survival. Ren *et al* (102) showed that altered glutamine usage in osteosarcoma elevates drug resistance by sustaining metabolic demands under stress conditions. In addition, research by Wang *et al* (103) highlighted the role of YAP1-mediated glutamine metabolism in osteosarcoma, indicating that disrupting this pathway could enhance therapeutic lethality. The branched-chain amino acids (BCAAs) are also implicated in osteosarcoma progression. Lin *et al* (104) found that angiopoietin like 4 regulates BCAA metabolism, presenting a potential target for disrupting metabolic communications in osteosarcoma. The metabolic flexibility conferred by amino acid utilization aids osteosarcoma cells in adapting to various environmental stressors.

Interactions with the TME. The TME significantly influences the metabolic landscape of osteosarcoma. A study has shown that metabolic gene expression correlates with immune microenvironment alterations, impacting clinical outcomes (105). Wu *et al* (106) explored the metabolic interplay between osteosarcoma cells and the immune microenvironment, suggesting that a thorough understanding of these interactions could lead to novel immunotherapy approaches. Moreover, copper metabolism, highlighted in recent work, affects both the immune landscape and the viability of osteosarcoma cells (107). Lin *et al* (108) emphasized the need to assess the copper-metabolism-related genes for further insights into osteosarcoma prognosis and treatment responses.

As the understanding of the metabolic alterations in osteosarcoma deepens, it brings forth exciting prospects for targeted metabolic interventions. The integration of multi-omics approaches is essential for elucidating the complex metabolic networks underlying osteosarcoma, potentially leading to personalized therapeutic strategies (93). Future research should continue to explore the interactions among different metabolic pathways and their implications in the TME, providing a foundation for innovative treatment modalities based on metabolic reprogramming.

4. Integrative multi-omics approaches

Combining genomic, transcriptomic, proteomic and metabolomic data. The integration of multi-omics data provides unique challenges and opportunities. Techniques such as machine learning, bioinformatics and systems biology are becoming essential for managing the vast datasets generated by each omics layer (10). Machine learning methods, particularly those based on supervised and unsupervised algorithms, can be employed to identify patterns and correlations among genomic, transcriptomic, proteomic and metabolomic profiles. These methods facilitate the extraction of meaningful insights that a single omics approach may overlook (10). Recent advancements in computational frameworks, such as weighted gene co-expression network analysis and pathway enrichment tools, have further enabled the identification of cross-omics interactions, enhancing the interpretability of complex datasets (109).

One illustrative case study is the combined omics analysis conducted by Lin *et al* (110), which investigated the molecular landscape of osteosarcoma. The study utilized RNA-seq data to identify differentially expressed genes, while proteomic profiling revealed novel proteins associated with disease progression. By integrating these findings with metabolomic data, they were able to identify specific metabolic pathways, such as glycolysis and lactate metabolism, that were altered in osteosarcoma. This integrative approach provided a more holistic understanding of the TME and its metabolic adaptation, highlighting the critical interplay between different molecular layers.

Table IV. Metabolomics findings in osteosarcoma research.

Author/s, year	Metabolites	Samples	Major findings	(Refs.)
Hu *et al*, 2022	Sphingolipid	Data from The Cancer Genome Atlas	Sphingolipid metabolism promotes osteosarcoma metastasis and worsens prognosis by activating notch signaling and angiogenesis pathways.	(95)
Cai *et al*, 2024	Lipid	Osteosarcoma tissues	Lipid metabolism promotes osteosarcoma proliferation and tumor growth by regulating lipogenic enzymes and the Akt/mTOR pathway, with RPARP-AS1 acting as a critical oncogenic lncRNA.	(96)
Bispo *et al*, 2023	Lipid	Osteosarcoma cells	Lipid metabolism influences osteosarcoma cell responses to chemotherapy by affecting membrane fluidity and triglyceride levels, with cisplatin enhancing apoptosis while Pd_2Spermine primarily modulates membrane properties.	(97)
Fritsche-Guenther *et al*, 2020	Fatty acid oxidation	A female osteosarcoma cell line	Fatty acid oxidation supports osteosarcoma metastasis by enhancing energy metabolism and metabolic flux in metastatic cells compared to benign counterparts.	(98)
Shen *et al*, 2023	Glycolysis	An osteosarcoma model	Glycolysis facilitates osteosarcoma progression by promoting a hypoxia-induced feedback loop involving circRNA Hsa_circ_0000566 and HIF-1α, enhancing the Warburg effect under hypoxic conditions.	(99)
Li *et al*, 2021	Glucose	miR-329-3p expression in osteosarcoma tissue	Glucose metabolism increases cisplatin resistance in osteosarcoma by enhancing LDHA activity, which is negatively regulated by miR-329-3p.	(100)
Wang *et al*, 2023	Glucose	Osteosarcoma cells	Glucose metabolism promotes osteosarcoma growth and cancer stem-like properties by facilitating aerobic glycolysis, which solasonine inhibits in an ALDOA-dependent manner.	(101)
Ren *et al*, 2024	Amino acids	A novel three-dimensional model of osteosarcoma cell lines	Amino acids, particularly glutamine, enhance osteosarcoma drug resistance by promoting metabolic changes in a three-dimensional microenvironment, which can be targeted to overcome resistance.	(102)
Wang *et al*, 2024	Glutamine	Single-cell transcriptomic data	Glutamine metabolism enhances the resistance of osteosarcoma to chemotherapy by activating the YAP1 pathway, which serves as a bypass mechanism against drug effects.	(103)
Lin *et al*, 2022	Branched-chain amino acids	Osteosarcoma samples and cell lines	BCAAs promote osteosarcoma progression by activating the mTOR signaling pathway in the absence of ANGPTL4, which negatively regulates BCAA catabolism.	(104)
Li *et al*, 2024	Copper	Osteosarcoma samples of patients	Copper metabolism, particularly through cuproptosis-related sphingolipid metabolism, regulates osteosarcoma progression and drug resistance by influencing immune cell infiltration and signaling pathways that govern cell migration and apoptosis.	(107)
Lin *et al*, 2023	Copper	Datasets GSE21257 including 50 osteosarcoma patients	Copper metabolism influences osteosarcoma progression and drug sensitivity by establishing a prognostic signature through CMRGs that correlates with immune activity and patient outcomes.	(108)

LDHA, lactate dehydrogenase-A; BCAAs, branched-chain amino acids; CMRGs, copper metabolism-related genes; HIF-1α, hypoxia-inducible factor 1α; YAP1, Yes-associated protein 1; mTOR, mammalian target of rapamycin; ALDOA, aldose reductase domain containing 1; ANGPTL4, angiopoietin-like 4; SDF-1, stromal cell-derived factor 1.

A recent multi-omics study employed single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics to dissect intratumoral heterogeneity in advanced osteosarcoma (111). The study identified distinct cellular subpopulations enriched in chemotherapy-resistant cells, characterized by upregulated glycolysis and PI3K/AKT pathway activity. Proteomic validation further confirmed the overexpression of glycolytic enzymes (e.g., LDHA) and AKT1 in these subpopulations, providing a mechanistic link between metabolic reprogramming and therapeutic resistance. This study exemplifies how integrative approaches can uncover actionable targets, such as combined inhibition of AKT and glycolysis, to overcome resistance.

Another compelling example involves a study by Jia *et al* (112), which integrated genomic, proteomic and metabolomic data to explore cuproptosis-related pathways in osteosarcoma. The authors identified a copper metabolism signature correlated with immune suppression and poor prognosis. Functional experiments demonstrated that copper chelators synergized with immune checkpoint inhibitors to enhance T-cell infiltration and reduce tumor growth in preclinical models. This translational application underscores the potential of multi-omics in guiding combination therapies tailored to metabolic-immune crosstalk.

Furthermore, Truong *et al* (113) mapped the differentiation trajectories of osteosarcoma cells using scRNA-seq and chromatin accessibility assays. They revealed that epigenetic remodeling of SOX9-driven chondrogenic pathways promotes metastatic niche formation. Complementary metabolomic profiling highlighted elevated glutamine utilization in metastatic cells, suggesting therapeutic vulnerability to glutaminase inhibitors. This study not only clarified the role of SOX9 in osteosarcoma progression but also demonstrated how multi-omics can bridge molecular mechanisms to therapeutic strategies.

Impact on understanding tumor biology and treatment options. Integrative multi-omics approaches significantly enhance our understanding of tumor biology, yielding crucial insights that can inform treatment decisions (114). For instance, the identification of specific genetic and proteomic alterations linked to signaling pathways, such as the mTOR and PI3K/AKT pathways, allows for the development of targeted therapies aimed at these pathways (115). Wang *et al* (103) combined CRISPR-Cas9 screens with proteomic profiling to identify YAP1-mediated glutamine addiction in chemotherapy-resistant osteosarcoma. They demonstrated that disrupting the YAP1-glutamine axis using the small-molecule inhibitor CA3 restored cisplatin sensitivity *in vivo*, highlighting the translational value of pathway-centric multi-omics.

Furthermore, studies leveraging integrative approaches can identify patient stratification markers, facilitating personalized treatment regimens (116). By categorizing patients based on distinct molecular signatures, clinicians can tailor therapies that specifically target the identified vulnerabilities of each tumor. For instance, patients exhibiting specific metabolic dysregulations could be treated with agents designed to exploit those metabolic weaknesses, potentially enhancing therapeutic efficacy (117).

Furthermore, the integrative analysis of multi-omics data can assist in predicting treatment responses and resistance mechanisms in osteosarcoma (107). Understanding the interactions between genetic alterations, changes in gene expression and alterations in protein levels can provide insights into why certain patients may not respond to conventional therapies (118). For instance, the presence of specific mutations alongside corresponding protein changes can indicate an adaptive resistance mechanism, signaling the need for alternative treatment options (118).

The integrative analysis of multi-omics data can also assist in predicting treatment responses and resistance mechanisms in osteosarcoma (104). For example, Audinot *et al* (119) utilized circulating tumor DNA sequencing and longitudinal metabolomics to monitor osteosarcoma evolution during chemotherapy. They identified recurrent mutations in TP53 and RB1 alongside increased kynurenine pathway activity as hallmarks of acquired resistance. Targeting these pathways with poly(ADP-ribose) polymerase inhibitors and indoleamine 2,3-dioxygenase 1 blockers in patient-derived xenografts (PDXs) significantly delayed relapse, illustrating the power of dynamic multi-omics monitoring.

In summary, integrative multi-omics approaches not only provide a comprehensive view of the complex biological landscape of osteosarcoma but also foster innovation in treatment strategies. The ability to couple genomic, transcriptomic, proteomic and metabolomic data through advanced computational techniques can lead to the discovery of novel biomarkers and therapeutic targets, ultimately guiding the implementation of precision medicine in osteosarcoma management.

5. Translational applications of multi-omics in osteosarcoma

The integration of multi-omics approaches encompassing genomics, transcriptomics, proteomics and metabolomics holds great promise in enhancing the diagnosis and management of osteosarcoma (Table V) (4). The targets identified through multi-omics strategies show significant potential in the diagnosis and treatment of osteosarcoma, providing direction for targeted therapy and combination therapy for this condition (Fig. 2).

Mutations in TP53 have been extensively studied as potential biomarkers. Various studies indicate that TP53 mutations are associated with aggressive disease and poor outcomes in osteosarcoma (120). For instance, a meta-analysis identified TP53 mutations as significant prognostic factors, suggesting their potential as biomarkers for patient stratification (121). Specific polymorphisms in TP53 have also been linked to susceptibility to osteosarcoma among certain populations, providing insights into genetic predisposition (122). MDM2, an antagonist of TP53, has emerged as another potential biomarker. Its overexpression has been implicated in various malignancies, including osteosarcoma. Research demonstrates that MDM2 overexpression can be detected via immunohistochemistry and correlates with lower survival rates, making it a valuable adjunct in the diagnosis and prognostic assessment of patients with osteosarcoma (123). Cyclin-dependent kinases (CDKs), particularly CDK4, have garnered attention as biomarkers in osteosarcoma. Overexpression of CDK4 is associated with resistance to conventional chemotherapy and

Table V. The clinical value of multi-omics findings in osteosarcoma.

Author/s, year	Biomarker	Clinical application	Expected effect	(Refs.)
Chen *et al*, 2016	TP53	Diagnosis	TP53 mutations serve as a significant prognostic marker indicating poor 2-year overall survival in patients with osteosarcoma.	(121)
Ru *et al*, 2015	TP53	Diagnosis	TP53 genetic mutations serve as significant indicators of osteosarcoma risk and prognosis in the Chinese population.	(122)
Jeon *et al*, 2015	CDK4 and MDM2	Diagnosis	CDK4 immunostaining may serve as a valuable diagnostic adjunct for low-grade central osteosarcoma, although its negative results do not exclude the diagnosis.	(123)
Iwata *et al*, 2021	CDK4	Diagnosis	CDK4 amplification and overexpression in osteosarcoma tumors serve as predictive biomarkers for resistance to conventional chemotherapy.	(124)
Schubert *et al*, 2022	CDK4/6	Diagnosis	CDK4/6 genomic aberrations in osteosarcoma indicate potential as therapeutic targets, though their correlation with treatment efficacy requires further evaluation.	(125)
Sun *et al*, 2016	EZH2	Diagnosis and treatment	EZH2 overexpression serves as a potential prognostic marker for aggressive behavior and poor outcomes in osteosarcoma, highlighting its role as a promising therapeutic target.	(126)
Nazarizadeh *et al*, 2021	Osteopontin	Diagnosis	Osteopontin shows promise as a sensitive and specific biomarker for diagnosing bone cancers and predicting their clinicopathological features.	(128)
Barris *et al*, 2018	ctDNA	Diagnosis	ctDNA analysis offers a promising non-invasive approach for detecting somatic alterations in osteosarcoma, which may aid in patient management and treatment monitoring.	(129)
Audinot *et al*, 2024	ctDNA	Diagnosis	ctDNA quantification enhances the prognostic stratification of osteosarcoma patients, serving as a significant independent prognostic factor alongside traditional clinical parameters.	(119)
Zhao *et al*, 2015	VEGF	Treatment	VEGF serves as a crucial target in osteosarcoma therapy, as its expression is directly regulated by miR-410, which, when overexpressed, inhibits tumor growth and promotes apoptosis in osteosarcoma cells.	(131)
Tawbi *et al*, 2017	VEGF	Treatment	VEGF-targeted therapy holds potential in bone sarcoma treatment, where it may enhance response rates and overall survival, particularly when combined with immunotherapy approaches like pembrolizumab.	(132)
Mickymaray *et al*, 2021	PI3K/AKT/ mTOR pathway	Treatment	The PI3K/AKT/mTOR pathway is a promising target in osteosarcoma therapy, as its downregulation by Rhaponticin effectively induces cytotoxic effects and apoptosis in osteosarcoma cells.	(134)
Lu *et al*, 2020	EZH2	Treatment	EZH2 represents a valuable therapeutic target in osteosarcoma treatment, as its regulation by miR-449a is linked to inhibited tumor progression and improved patient prognosis.	(135)
Chen *et al*, 2023	EZH2	Treatment	EZH2 is a key target in osteosarcoma therapy, as its downregulation by capsaicin significantly inhibits cancer stemness and metastatic potential, potentially improving patient outcomes.	(136)

Table V. Continued.

Author/s, year	Biomarker	Clinical application	Expected effect	(Refs.)
Liu *et al*, 2019	PD-1/PD-L1 axis	Treatment	Targeting the PD-1/PD-L1 axis enhances the effectiveness of cisplatin chemotherapy in osteosarcoma, improving tumor control and promoting apoptosis in cancer cells.	(137)
Yoshida *et al*, 2019	PD-L1	Treatment	PD-L1 expression in osteosarcoma correlates with early metastatic formation and may serve as a predictive marker for the effectiveness of anti-PD-1 therapy, highlighting its potential in targeted treatment strategies.	(138)
Davis *et al*, 2020	PD-1/PD-L1	Treatment	Targeting PD-1 with nivolumab demonstrates a favorable safety profile in children and young adults, indicating its potential as a therapeutic option for osteosarcoma and other pediatric malignancies, particularly in combination therapies.	(139)
Zhou *et al*, 2018	CDK4	Treatment	CDK4 represents a promising therapeutic target in osteosarcoma, as its inhibition significantly suppresses tumor growth and migratory potential, and induces apoptosis in cancer cells.	(140)
Oshiro *et al*, 2021	CDK4/6	Treatment	The combination of CDK4/6 and mTOR inhibitors shows promising therapeutic potential for treating doxorubicin-resistant osteosarcoma by significantly reducing tumor volume and inducing tumor necrosis.	(141)

CDK, cyclin-dependent kinases; MDM2, mouse double minute 2; EZH2, zeste homologue 2; ctDNA, circulating tumor DNA; VEGF, vascular endothelial growth factor; PD-1, programmed death-1; PD-L1, programmed death-ligand 1.

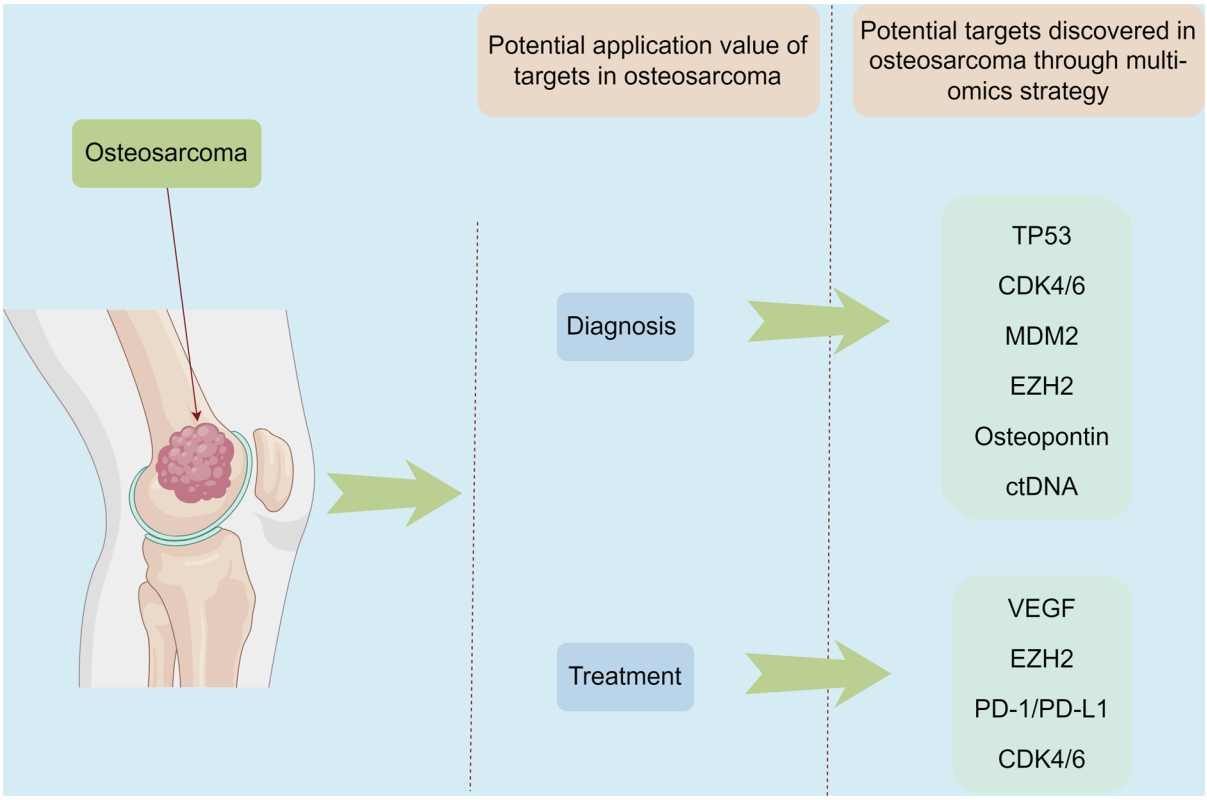

Figure 2. Potential targets identified through multi-omics strategies for osteosarcoma diagnosis and treatment. It is highlighted how genomics, transcriptomics, proteomics and metabolomics collectively contribute to the identification of biomarkers and therapeutic targets. These integrated insights facilitate the development of precision medicine approaches tailored to the molecular profile of individual tumors. The figure was generated using Figdraw (https://www.figdraw.com). CDK, cyclin-dependent kinases; MDM2, mouse double minute 2; EZH2, zeste homologue 2; ctDNA, circulating tumor DNA; VEGF, vascular endothelial growth factor; PD-1, programmed death-1; PD-L1, programmed death-ligand 1.

correlates with aggressive disease features (124). Furthermore, CDK4/6 inhibitors are being explored as potential therapeutic options, thus reinforcing the clinical relevance of CDK4 as a biomarker in osteosarcoma (125).

Enhancer of zeste homologue 2 (EZH2), a histone methyltransferase, has been implicated in osteosarcoma progression and poor prognosis. High levels of EZH2 expression have been associated with advanced disease stages and metastasis, establishing its role as a prognostic marker (126). Furthermore, EZH2 inhibitors are being evaluated in clinical trials, showing promise in targeted therapy approaches for osteosarcoma (127). Osteopontin has been identified as a significant serum biomarker for osteosarcoma. Elevated levels of osteopontin are associated with poorer survival outcomes, making it a potential marker for disease monitoring and therapy response (128). Its measurement in biofluids may provide real-time insights into the disease state. The use of circulating tumor DNA (ctDNA) represents a breakthrough in the non-invasive monitoring of osteosarcoma. ctDNA analysis enables the detection of tumor-specific mutations and informs about minimal residual disease and the risk of recurrence (129). Recent studies have validated the clinical utility of ctDNA quantification in predicting outcomes, indicating its integration into routine management protocols (119).

Recent advancements in metabolomics have identified various metabolites that exhibit significant alterations in patients with osteosarcoma compared to healthy controls. Specific metabolic signatures associated with tumor presence and progression may serve as novel diagnostic biomarkers, although research in this area is still in its infancy (130). Multi-omics technologies have significantly expanded the understanding of osteosarcoma, identifying various biomarkers with diagnostic and prognostic potential. Integrating these biomarkers into clinical workflows may facilitate earlier diagnosis, enhance personalized treatment strategies and ultimately improve patient outcomes. Future research should focus on validating these biomarkers in larger cohorts and elucidating their mechanisms of action, propelling the field toward precision medicine in osteosarcoma management.

VEGF has emerged as a pivotal biomarker in osteosarcoma due to its role in tumor angiogenesis. Studies have demonstrated that inhibition of VEGF not only reduces tumor growth but also enhances the sensitivity of osteosarcoma cells to therapeutic agents. For instance, silencing VEGF through microRNAs, such as miR-410, has been shown to suppress proliferation and invasion of osteosarcoma cells (131). Furthermore, the application of anti-VEGF therapies, such as bevacizumab, in combination with other agents may provide a synergistic effect, as evidenced in recent clinical trials (132,133).

The PI3K/AKT/mTOR pathway is frequently dysregulated in osteosarcoma, making it a suitable target for intervention. Various studies have explored this pathway's role in cell survival and proliferation. For instance, the natural compound rhaponticin was shown to inhibit osteosarcoma via suppression

of the PI3K-Akt-mTOR signaling axis (134). Furthermore, selective inhibition of EZH2 has been demonstrated to effectively regulate the PI3K/AKT signaling pathway, impacting cancer cell behavior (135,136).

The blockade of immune checkpoints, particularly programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1), has been explored in osteosarcoma and demonstrates significant therapeutic potential. Research indicates that combining PD-1/PD-L1 inhibitors with traditional chemotherapeutics can enhance anti-tumor efficacy by overcoming immune escape mechanisms (137,138). Furthermore, the combination of immune checkpoint blockade and T-cell therapies has shown promising results in clinical settings (139).

The CDK pathway, specifically CDK4/6, has gained attention as a therapeutic target due to its involvement in cell cycle regulation in osteosarcoma (140). Targeting CDK4/6 has been shown to enhance the effects of chemotherapy and may help in the management of drug-resistant osteosarcoma variants (125,141).

In conclusion, multi-omics approaches have significantly advanced the understanding of the molecular underpinnings of osteosarcoma. By translating these findings into clinical applications, it may be possible to better harness targeted therapies, ultimately improving patient outcomes in this challenging malignancy. Continuous research and clinical trials will be paramount to validate these strategies and ensure their efficacy in the broader patient population.

6. Future directions

One of the most pressing needs in osteosarcoma research is the establishment of collaborative research networks that promote data sharing among institutions worldwide (142). The heterogeneity of osteosarcoma, combined with the complexity of multi-omics data, necessitates a collaborative approach to facilitate large-scale studies and the development of robust databases that integrate genomic, transcriptomic, proteomic and metabolomic information (13).

Current efforts, such as the Pediatric Cancer Genome Project, provide a framework for collaborative research but need to be expanded to include international participants and diverse patient populations (143). Furthermore, data sharing platforms, such as the European Genome-phenome Archive (https://ega-archive.org) and cBioPortal (https://www.cbioportal.org), play a crucial role in providing researchers access to valuable datasets, fostering collaborations that can lead to meaningful discoveries in osteosarcoma research (144).

By encouraging multi-institutional collaborations, it may be possible to facilitate the standardization of omics data collection protocols, ensuring consistency and reproducibility among disparate studies. As multi-omics research evolves, a shared commitment to open science will enhance the overall quality of research findings and enable faster translation into clinical applications. The future of multi-omics in osteosarcoma research is closely tied to the rapid advancements in emerging technologies, particularly single-cell omics (113). Single-cell sequencing technologies allow for the analysis of individual cells within a tumor, providing unprecedented insights into tumor heterogeneity, cell lineage and microenvironment interactions that traditional bulk analysis cannot achieve (145).

These technologies hold the potential to uncover specific cell populations that drive tumor growth and metastasis, which can lead to the identification of novel biomarkers and therapeutic targets (146). Recent studies employing scRNA-seq in osteosarcoma have revealed distinct cellular subpopulations that contribute to the TME's immunosuppressive nature, potentially guiding the development of more effective immunotherapy strategies (111,147,148). Furthermore, integrating single-cell proteomics and metabolomics with transcriptomics can help create a multi-layered understanding of the molecular dynamics within tumors (111). Future initiatives should focus on standardizing these methodologies and developing comprehensive platforms that seamlessly integrate heterogeneous single-cell data for further analysis.

Longitudinal studies that utilize patient-derived samples are critical for advancing the current understanding of osteosarcoma's progression and treatment responses. Monitoring tumors over time through the collection of biopsies, blood samples for ctDNA and other biofluids can provide valuable insights into the temporal dynamics of genomic and phenotypic alterations (149). Such studies can enhance our understanding of how tumors evolve under specific therapies, allowing for the identification of resistance mechanisms and the development of adaptive treatment approaches. For instance, a prospective trial analyzing ctDNA in patients with osteosarcoma demonstrated that detecting mutations associated with chemotherapy resistance provided critical insights for treatment modifications (119).

Furthermore, incorporating PDXs and organoids derived from osteosarcoma tumors into research initiatives allows for a more accurate representation of tumor biology in the laboratory (150,151). These models can be utilized for drug screening and validation of treatment strategies before clinical implementation, ultimately promoting personalized therapy. To successfully implement these initiatives, securing funding and collaboration from stakeholders, including academic institutions, pharmaceutical companies and patient advocacy groups, will be vital. Engaging patients in the research process, ensuring their informed consent and privacy are respected, will further promote patient-centric research endeavors that align with modern ethical standards.

The future directions and initiatives in multi-omics research for osteosarcoma promise to enhance the current understanding and management of this complex disease. By fostering collaborative research and data sharing, harnessing the potential of emerging technologies such as single-cell omics, and implementing longitudinal studies using patient-derived samples, it may be possible to pave the way for precision medicine that caters to the individual needs of patients with osteosarcoma. As these initiatives evolve, they will play a vital role in overcoming the challenges currently faced by osteosarcoma research and ultimately improve patient outcomes.

7. Conclusion

In conclusion, the integration of multi-omics approaches holds transformative potential for osteosarcoma research and treatment, allowing for a comprehensive understanding of tumor heterogeneity and the development of targeted therapies. By

elucidating the intricate molecular networks underlying osteosarcoma, it may be possible to enhance early diagnosis and personalize treatment strategies, ultimately improving patient outcomes. It is imperative for clinicians and researchers to collaborate and advocate for the incorporation of multi-omics into clinical practice to advance precision medicine and offer hope to patients with osteosarcoma.

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Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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