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. It generally arises in adolescents and young adults, peaking between the ages of and years, during periods of rapid skeletal growth. Epidemiologically, osteosarcoma has an annual incidence of 3-4 cases per million individuals in the general population, with a notable male predominance Geographic variations in incidence have been observed, with certain studies indicating higher rates in African populations and lower frequencies in Asian cohorts. Recent advancements in genomic studies have identified possible genetic risk factors, including mutations in the tumor protein (TP) gene linked to Li-Fraumeni syndrome. 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.

The current standard of care for osteosarcoma primarily involves a multidisciplinary approach, combining neoadjuvant chemotherapy, surgical resection and adjuvant chemotherapy. High-dose multi-agent chemotherapy, typically including doxorubicin, cisplatin and methotrexate, aims to shrink tumors before surgery and eliminate micrometastatic disease postoperatively. 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. Furthermore, the potential for severe adverse effects from aggressive chemotherapy regimens poses significant challenges to patient quality of life. 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. This concept facilitates the understanding of the complex molecular interplay driving tumorigenesis, progression and response to therapy. 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. 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. 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.

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.

. 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. Key mutations commonly observed in osteosarcoma include alterations in the TP, retinoblastoma protein (RB) and mouse double minute 2(MDM2) genes 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. Mutations in RB, another critical tumor suppressor, disrupt the Rb-E F pathway, promoting uncontrolled cellular proliferation and contributing to tumor growth. Meanwhile, MDM overexpression results in the degradation of p, further exacerbating the dysregulation of apoptosis and cell survival.

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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 (). 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 .

Role of the tumor microenvironment (TME)

The TME plays a crucial role in shaping the behavior and progression of osteosarcoma (). Comprising various cell types, including immune cells, fibroblasts, endothelial cells and extracellular matrix (ECM) components, the TME significantly influences tumor growth and metastasis . 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. Certain soluble factors within the TME can also affect the behavior of osteosarcoma cells . 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 . 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 . 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 . 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 (). 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 . 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 . The presence of these resistant subpopulations can lead to incomplete responses to treatment, ultimately resulting in unfavorable prognostic outcomes . Furthermore, the dynamic nature of tumor evolution complicates the understanding of treatment resistance . 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 . Integrating multi-omics approaches to profile the genetic, transcriptomic and proteomic landscapes of osteosarcoma will provide insights into the mechanisms of heterogeneity and resistance, paving the way for the development of targeted therapeutic strategies tailored to specific tumor characteristics . 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 . 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 . As we move towards precision medicine, a thorough comprehension of these complexities will be indispensable in addressing the limitations of current treatment paradigms. . Overview of multi-omics approaches

Genomics

Recent advances in whole-genome sequencing (WGS) have significantly enhanced our understanding of the genomic landscape of osteosarcoma . WGS allows for the identification of genetic alterations, comprehensively analyzing both coding and non-coding regions of the genome . Studies have identified critical driver mutations in key genes such as TP , RB , and MDM , which play pivotal roles in tumor development and progression.

Mutations in key tumor suppressor genes

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

Amplification of the MDM gene, which encodes a protein that inhibits TP, is present in 10-15 % of osteosarcoma cases. Increased MDM expression facilitates the bypass of TP-mediated apoptosis, aiding in tumor growth and resistance to therapies. Its role in the differentiation of osteosarcoma from benign lesions highlights its diagnostic significance. The PIK/AKT signaling pathway is frequently activated in osteosarcoma. For instance, a study on C-type lectin domain family member A demonstrated that its suppression led to reduced cell proliferation through the AKT serine/threonine kinase (AKT)/mammalian target of rapamycin (mTOR) axis. Furthermore, ménage à trios has been shown to facilitate lung metastasis via upregulation of AKT expression, underscoring the pathway's role in metastatic spread. Alterations in growth factor receptors

The fibroblast growth factor receptor (FGFR) signaling pathway has gained attention due to its involvement in osteosarcoma progression. The FGFR signaling pathway, particularly FGFR, plays a critical role in the progression of osteosarcoma. Overexpression of FGF and FGFRs has been linked to increased cell migration, invasion and metastasis, notably through intercellular adhesion molecule- expression. Furthermore, nuclear FGFR contributes to radiation resistance by inducing cell survival mechanisms, such as G- 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. Epigenetic modifications

Aberrant epigenetic modifications have been identified in osteosarcoma that may contribute to its aggressiveness. For instance, alterations in histone markers, such as HKme, have been noted in various osteosarcoma subtypes, indicating heterogeneous disease behavior. 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-FGFR, which present new therapeutic targets. 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 MDM overexpression may prompt the use of specific MDM inhibitors, tailoring treatment to the genetic landscape of the tumor.

The totality of genomic research on osteosarcoma illustrates a landscape of intricate genetic alterations that drive tumorigenesis. The mutations in tumor suppressor genes like TP and RB, the amplification of oncogenes such as MDM and AKT, and disturbances to growth factor signaling pathways underscore the heterogeneous nature of this malignancy. 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. 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 TP gene are prevalent in numerous cancers, including osteosarcoma. Research indicates that TP missense mutations are associated with longer survival in a canine model of osteosarcoma, suggesting a potential survival advantage linked to specific mutations. Furthermore, the impairment of rigid sensing due to mutant TP gain-of-function mutations has been noted, implicating TP in the modulation of cellular mechanics and tumor progression. 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. 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. The downregulation of GATA binding protein 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. 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. The dual inhibition of VEGF and survivin has exhibited proliferation inhibition and induced apoptosis in osteosarcoma cells, thereby presenting a promising therapeutic strategy. In addition, the splicing factor YBX promotes osteosarcoma progression by upregulating VEGF and downregulating its anti-angiogenic isoform VEGF. Hypoxia-inducible factor α (HIF-α) mediates several processes involved in osteosarcoma, including cellular response to hypoxia and metabolism. Of note, HIF-α-mediated augmentation of certain microRNAs facilitates proliferation and metastasis, reflecting its role in EMT pathways. Furthermore, the interplay between HIF-α and mTOR signaling pathways is critical, as demonstrated by the synergistic anti-tumor activity of ginsenoside Rg alongside doxorubicin. Cell migration and invasion

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

The integration of transcriptomic data in understanding the biology of osteosarcoma heralds a shift towards precision medicine approaches. 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 TP, HIF-α and Myc provides a framework for developing targeted therapies that address the unique characteristics of each patient's tumor. 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. 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. 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. 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

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-α and CXCR. Chang et al elucidate that Nε-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 CXCR has been tightly correlated with disease progression and poor prognosis in osteosarcoma. Specifically, CXCR signaling has been shown to facilitate myeloid-derived suppressor cell accumulation, which can blunt responses to immunotherapy. Furthermore, CXCR blockade has been proven to sensitize osteosarcoma cells to doxorubicin through autophagic cell death induction and inhibition of the PIK-Akt-mTOR pathway. HIF-α, a key regulator in the tumor response to hypoxic conditions, enhances osteosarcoma proliferation and metastasis through various proteins, such as matrilin (MATN). HIF-α mediates the expression of MATN, promoting progression, indicating the dual role of hypoxic signaling in metabolic reprogramming and tumor aggressiveness.

Metastasis-related proteins

Metastasis is a complex biological process that involves multiple signaling pathways, with several proteins acting as key facilitators. MMPs, particularly MMP- and MMP-, are crucial for ECM degradation, contributing to metastasis. Overexpression of mucin (MUC) has been associated with enhanced osteosarcoma cell proliferation and invasiveness via the Wnt/β-catenin signaling pathway and regulated through MMP- and MMP-. A study indicated that sirtuin negatively regulates proliferation and invasion in osteosarcoma by targeting N-cadherin, 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. Proteins such as survivin and Livin have been highlighted as important mediators of apoptosis resistance in osteosarcoma. MUC was shown to promote cell proliferation through the Livin protein, linking the Wnt/β-catenin pathway with survival. The B-cell lymphoma- (Bcl-) inhibitor AT- has demonstrated efficacy in inhibiting osteosarcoma growth in preclinical models. Further exploration of how survival pathways modulated by Bcl- 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. A study indicated that lysine-specific demethylase B-mediated histone demethylation of lactate dehydrogenase A (LDHA) promotes lung metastasis in osteosarcoma. This highlights the role of LDHA in shaping the metabolic landscape. Furthermore, N-acetyltransferase -mediated acC acetylation of LDHA was shown to upregulate glycolytic metabolism in osteosarcoma. Aerobic glycolysis: The CXCR-CARM-YAP axis has been found to be critical in regulating glucose metabolism, linking aerobic glycolysis to chemoresistance. 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 (TRIM) offers a potential therapeutic strategy by destabilizing RACK and inactivating MEK/ERK signaling, demonstrating its role in osteosarcoma progression. Evidence suggests that Zyxin and TRIM restrict proliferation and metastasis via diverse signaling pathways, providing promising avenues for therapeutic explorations. The splicing factor Y-box binding protein (YBX) has been identified as a promoter of osteosarcoma progression through the upregulation of VEGF. Intervening in YBX 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. These proteins offer promising targets for new therapeutic strategies, directing future research toward enhancing targeted therapies and personalized medicine approaches for osteosarcoma. As 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. 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 .

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. For instance, Hu et al 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 , can regulate lipid metabolism, thus promoting osteosarcoma progression. Additionally, Bispo et al have shown that novel metal-based drugs can impact lipid metabolism in MG-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 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 noted that circular RNAs, such as Hsa_circ_, promote glycolysis and contribute to osteosarcoma progression by feedback regulation on HIF-α. Similarly, Li et al indicated that aberrations in glucose metabolism, specifically through the upregulation of LDHA by miR--, 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 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 showed that altered glutamine usage in osteosarcoma elevates drug resistance by sustaining metabolic demands under stress conditions. In addition, research by Wang et al highlighted the role of YAP-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 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. Wu et al 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. Lin et al 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. 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.

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. 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. 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.

One illustrative case study is the combined omics analysis conducted by Lin et al, 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.

A recent multi-omics study employed single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics to dissect intratumoral heterogeneity in advanced osteosarcoma. The study identified distinct cellular subpopulations enriched in chemotherapy-resistant cells, characterized by upregulated glycolysis and PIK/AKT pathway activity. Proteomic validation further confirmed the overexpression of glycolytic enzymes (e.g., LDHA) and AKT 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, 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 mapped the differentiation trajectories of osteosarcoma cells using scRNA-seq and chromatin accessibility assays. They revealed that epigenetic remodeling of SOX-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 SOX 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. For instance, the identification of specific genetic and proteomic alterations linked to signaling pathways, such as the mTOR and PIK/AKT pathways, allows for the development of targeted therapies aimed at these pathways. Wang et al combined CRISPR-Cas screens with proteomic profiling to identify YAP-mediated glutamine addiction in chemotherapy-resistant osteosarcoma. They demonstrated that disrupting the YAP-glutamine axis using the small-molecule inhibitor CA 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. 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.

Furthermore, the integrative analysis of multi-omics data can assist in predicting treatment responses and resistance mechanisms in osteosarcoma. 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. For instance, the presence of specific mutations alongside corresponding protein changes can indicate an adaptive resistance mechanism, signaling the need for alternative treatment options.

The integrative analysis of multi-omics data can also assist in predicting treatment responses and resistance mechanisms in osteosarcoma. For example, Audinot et al utilized circulating tumor DNA sequencing and longitudinal metabolomics to monitor osteosarcoma evolution during chemotherapy. They identified recurrent mutations in TP and RB alongside increased kynurenine pathway activity as hallmarks of acquired resistance. Targeting these pathways with polyADP-ribose polymerase inhibitors and indoleamine ,-dioxygenase 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.

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. 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 .

Mutations in TP have been extensively studied as potential biomarkers. Various studies indicate that TP mutations are associated with aggressive disease and poor outcomes in osteosarcoma. For instance, a meta-analysis identified TP mutations as significant prognostic factors, suggesting their potential as biomarkers for patient stratification. Specific polymorphisms in TP have also been linked to susceptibility to osteosarcoma among certain populations, providing insights into genetic predisposition. MDM, an antagonist of TP, has emerged as another potential biomarker. Its overexpression has been implicated in various malignancies, including osteosarcoma. Research demonstrates that MDM 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. Cyclin-dependent kinases (CDKs), particularly CDK, have garnered attention as biomarkers in osteosarcoma. Overexpression of CDK is associated with resistance to conventional chemotherapy and correlates with aggressive disease features. Furthermore, CDK/ inhibitors are being explored as potential therapeutic options, thus reinforcing the clinical relevance of CDK as a biomarker in osteosarcoma.

Enhancer of zeste homologue (EZH), a histone methyltransferase, has been implicated in osteosarcoma progression and poor prognosis. High levels of EZH expression have been associated with advanced disease stages and metastasis, establishing its role as a prognostic marker. Furthermore, EZH inhibitors are being evaluated in clinical trials, showing promise in targeted therapy approaches for osteosarcoma. 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. 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. Recent studies have validated the clinical utility of ctDNA quantification in predicting outcomes, indicating its integration into routine management protocols.

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. 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-, has been shown to suppress proliferation and invasion of osteosarcoma cells. 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. 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. Furthermore, selective inhibition of EZH has been demonstrated to effectively regulate the PI3K/AKT signaling pathway, impacting cancer cell behavior.

The blockade of immune checkpoints, particularly programmed death- (PD-)/programmed death-ligand (PD-L), has been explored in osteosarcoma and demonstrates significant therapeutic potential. Research indicates that combining PD-/PD-L inhibitors with traditional chemotherapeutics can enhance anti-tumor efficacy by overcoming immune escape mechanisms. Furthermore, the combination of immune checkpoint blockade and T-cell therapies has shown promising results in clinical settings. The CDK pathway, specifically CDK/, has gained attention as a therapeutic target due to its involvement in cell cycle regulation in osteosarcoma. Targeting CDK/ has been shown to enhance the effects of chemotherapy and may help in the management of drug-resistant osteosarcoma variants. 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. .

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. 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. 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. Furthermore, data sharing platforms, such as the European Genome-phenome Archive and cBioPortal, play a crucial role in providing researchers access to valuable datasets, fostering collaborations that can lead to meaningful discoveries in osteosarcoma research. 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. 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. 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. 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. Furthermore, integrating single-cell proteomics and metabolomics with transcriptomics can help create a multi-layered understanding of the molecular dynamics within tumors.

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. 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. Furthermore, incorporating PDXs and organoids derived from osteosarcoma tumors into research initiatives allows for a more accurate representation of tumor biology in the laboratory. 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. .

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.

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