Abstract

Osteosarcoma is recognized as the most prevalent primary bone malignancy, primarily affecting children and adolescents. It is characterized by its aggressive behavior and high metastatic potential, which often leads to poor patient outcomes. Despite advancements in surgical techniques and chemotherapy regimens, the prognosis for patients with osteosarcoma remains unsatisfactory, with survival rates plateauing over the past few decades. A significant barrier to effective treatment is the development of chemotherapy resistance, which complicates the management of the disease and contributes to high rates of recurrence. This review article aims to provide a comprehensive overview of recent advancements in osteosarcoma therapy, particularly in overcoming chemotherapy resistance. We begin by discussing the current standard treatment modalities, including surgical resection and conventional chemotherapy agents such as methotrexate, doxorubicin, and cisplatin.

While these approaches have been foundational in managing osteosarcoma, they are often limited by adverse effects and variability in efficacy among patients. To address these challenges, we explore novel pharmacological strategies that aim to enhance treatment outcomes. This includes targeted therapies focusing on specific molecular alterations in osteosarcoma cells and immunotherapeutic approaches designed to harness the body's immune system against tumors. Additionally, we review innovative drug delivery systems that aim to improve the bioavailability and efficacy of existing treatments while minimizing toxicity. The review also assesses the mechanisms underlying chemotherapy resistance, such as drug efflux mechanisms, altered metabolism, and enhanced DNA repair pathways. By synthesizing current research findings, we aim to highlight the potential of new therapeutic agents and strategies for overcoming these resistance mechanisms. Ultimately, this article seeks to inform future research directions and clinical practices, underscoring the need for continued innovation in treating osteosarcoma to improve patient outcomes and survival rates.

Keywords: osteosarcoma, chemotherapy agents, chemotherapy resistance, immunotherapy, novel therapies, targeted therapy

1. Introduction

1.1. Overview of Osteosarcoma

Osteosarcoma (OS) is the most common primary bone malignancy, primarily affecting children, adolescents, and young adults. This aggressive tumor typically arises in the metaphysis of long bones, particularly around the knee and shoulder. The incidence of osteosarcoma peaks during periods of rapid skeletal growth, making it particularly relevant in pediatric and adolescent populations. Although the exact etiology remains largely unknown, several genetic and environmental factors have been implicated in its development. Notably, hereditary conditions such as Li-Fraumeni syndrome, hereditary retinoblastoma, and Rothmund–Thomson syndrome significantly increase the risk of osteosarcoma. Additionally, environmental factors, including previous radiation exposure and specific chemical exposures, may also contribute to its onset.

The clinical presentation of osteosarcoma can vary, but patients often initially present with localized pain and swelling at the tumor site. Several patients are first managed conservatively for suspected injury or trauma with no advancements. As the tumor progresses, systemic symptoms such as fever, weight loss, and fatigue may develop. Radiological imaging techniques, including X-rays, magnetic resonance imaging (MRI), and computed tomography (CT) scans, are essential for diagnosis and staging, assessing tumor size, location, and potential metastasis. Additionally, bone scans can also be employed to detect skeletal involvement outside the primary site. Histopathological examination of biopsy specimens is crucial for confirming the diagnosis, where characteristic patterns of osteoblastic, chondroblastic, or fibroblastic differentiation can be identified.

1.2. Standard Treatment Modalities

The treatment of osteosarcoma has evolved significantly over the years. The standard approach now involves a combination of surgical resection and chemotherapy, which has been foundational in improving patient outcomes. Surgical intervention typically aims for complete tumor removal, often necessitating limb-salvage procedures or, in some cases, amputation. The introduction of neoadjuvant chemotherapy has transformed the management of osteosarcoma by facilitating tumor shrinkage before surgery, allowing for more conservative surgical approaches and potentially enhancing survival rates.

The chemotherapy regimen commonly employed for osteosarcoma includes agents such as methotrexate, doxorubicin, and cisplatin. These drugs work by targeting rapidly dividing cancer cells, but they are not without significant side effects. Adverse effects such as myelosuppression, gastrointestinal disturbances, and cardiotoxicity can severely impact a patient’s quality of life and complicate treatment adherence. Furthermore, the efficacy of these agents varies among patients, with some experiencing robust responses while others exhibit minimal benefit. This variability is a significant barrier to achieving consistent treatment outcomes.

1.3. The Need for New Treatment Approaches

Despite advancements in surgical techniques and chemotherapy regimens, the prognosis for patients with osteosarcoma remains unsatisfactory, particularly in cases with metastatic disease. The five-year survival rate for localized osteosarcoma is approximately 60–70%, but this figure drops significantly to about 20–30% for patients with metastasis at the time of diagnosis. A substantial barrier to effective treatment is developing chemotherapy resistance, which complicates the management of the disease and contributes to high rates of recurrence. The mechanisms underlying this resistance are multifaceted and can include drug efflux, enhanced DNA repair mechanisms, and alterations in cellular metabolism.

Research has identified several key factors within the tumor microenvironment (TME) that contribute to developing chemotherapy resistance. The TME is a complex ecosystem composed of various cell types, including immune cells, fibroblasts, and endothelial cells, along with extracellular matrix components. Interactions between cancer cells and their microenvironment can influence tumor growth, metastasis, and response to therapy. For instance, cancer-associated fibroblasts (CAFs) can secrete growth factors and extracellular matrix proteins that promote tumor survival and limit the effectiveness of chemotherapy. Additionally, immune cells in the TME can adopt pro-tumorigenic roles, further complicating

treatment outcomes. Moreover, adaptive mechanisms of the TME, such as extracellular matrix remodeling and hypoxia adaptation, create additional barriers to effective therapy.

To address the challenges posed by chemotherapy resistance, there is a growing interest in novel pharmacological strategies to enhance treatment outcomes for osteosarcoma patients. Targeted therapies focusing on specific molecular alterations present in osteosarcoma cells have emerged as promising alternatives to conventional chemotherapy. These therapies exploit the unique vulnerabilities of osteosarcoma cells by targeting the signaling pathways and genetic mutations driving tumor growth. For instance, inhibitors of the mammalian target of rapamycin (mTOR) pathway and receptor tyrosine kinases (RTKs) have shown potential in preclinical and early clinical studies.

Immunotherapeutic approaches are gaining traction in the treatment of osteosarcoma. By harnessing the body's immune system, these therapies aim to enhance the antitumor response and overcome the immunosuppressive effects of the tumor microenvironment. Chimeric antigen receptor (CAR) T-cell therapy and immune checkpoint inhibitors have demonstrated promise in other malignancies, and ongoing research is investigating their potential applications in osteosarcoma. Furthermore, the development of personalized immunotherapies targeting specific tumor antigens represents a rapidly evolving area of research with significant implications for improving patient outcomes.

In addition to targeted therapies and immunotherapy, innovative drug delivery systems are being explored to enhance treatment efficacy and minimize toxicity. In particular, nanoparticle-based delivery systems can improve the targeted delivery of chemotherapy drugs to tumor sites, thereby reducing systemic exposure and associated side effects. Various nanocarriers, including liposomes, polymeric nanoparticles, dendrimers, and micelles, have been developed to enhance drug delivery by leveraging the enhanced permeability and retention (EPR) effect for tumor targeting. Furthermore, active targeting schemes, including ligand modifications, enhance the specificity of these nanocarriers. Clinical trials have shown promising results, leading to approvals for formulations like Mepact®. While these advancements significantly improve drug bioavailability and reduce systemic side effects and drug resistance, challenges remain in translating these technologies into clinical practice, including issues of nanoparticle stability, scalability, and regulatory hurdles.

The mechanisms underlying chemotherapy resistance in osteosarcoma are complex and multifactorial. Understanding these mechanisms is critical for the development of effective strategies to overcome resistance. Drug efflux mechanisms, where cancer cells utilize transport proteins to pump out chemotherapeutic agents, can significantly diminish drug efficacy. Additionally, alterations in drug metabolism, including increased expression of detoxifying enzymes, can further contribute to resistance. Enhanced DNA repair pathways, which allow cancer cells to repair the damage caused by chemotherapy, also play a pivotal role in resistance development.

We aim to highlight new therapeutic agents' potential and strategies for overcoming resistance mechanisms by integrating current research findings. Our review provides a comprehensive overview of recent advancements in osteosarcoma therapy, emphasizing the importance of addressing chemotherapy resistance to improve patient outcomes. We will

explore the interplay between the tumor microenvironment and treatment efficacy and the role of novel pharmacological approaches in enhancing therapeutic responses.

This article aspires to inform future research directions and clinical practices, underscoring the need for continued innovation in the treatment of osteosarcoma. As we strive to improve patient outcomes and survival rates, a deeper understanding of the molecular underpinnings of osteosarcoma and its microenvironment will be essential in guiding the development of more effective therapies.

2. Chemotherapy Resistance in Osteosarcoma: Therapeutic Implications

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and young adults, characterized by high metastatic potential and poor prognosis for metastatic cases. Standard treatment for osteosarcoma typically includes a combination of surgical intervention and multi-agent chemotherapy. The surgical approach focuses on removing the tumor with adequate margins while maintaining the functionality of the affected limb. Pre-operative and post-operative chemotherapy regimens, which include methotrexate, doxorubicin, and cisplatin (MAP), are designed to reduce tumor size before surgery and to eliminate any residual tumor cells afterward.

However, systemic chemotherapy for OS faces significant challenges due to its invasive nature and the pain it causes, which can significantly impact the quality of life of patients. One major issue is that systemic drugs often struggle to reach cancer cells located far from blood vessels, resulting in low drug concentrations in the affected bone. This problem is exacerbated by factors such as drug instability in the bloodstream, protein binding, and clearance by liver cells, which all contribute to toxicity related to the doses given. As a result, high systemic doses are frequently required, which can lead to severe adverse effects, including myelosuppression, hepatotoxicity, cardiotoxicity, and potentially fatal central nervous system complications. Chemotherapy resistance in OS remains a significant obstacle, with over 30% of patients exhibiting resistance to current treatments or experiencing severe side effects, ultimately leading to disease progression and increased mortality. To address these challenges, recent studies have concentrated on developing advanced drug delivery systems aimed at enhancing the effectiveness of chemotherapy while minimizing side effects. Additionally, understanding the mechanisms of chemotherapy resistance is essential for creating new therapeutic strategies to improve patient outcomes.

2.1. Mechanisms of Chemotherapy Resistance

Chemotherapeutic resistance is an ongoing problem in osteosarcoma therapy driven by multidimensional biological pathways facilitating survival and adaptation to chemostress in tumor cells. Among these essential pathways is overexpression of ATP-binding cassette (ABC) transporters to actively remove chemotherapeutic drugs from cancer cells; changes in metabolism through enzymes such as cytochrome P450 (CYP) and glutathione S-transferases (GSTs) to modulate inactivation and clearance of drugs; augmented DNA repair pathways, including base excision repair (BER) and nucleotide excision repair (NER) pathways, to reverse therapy-induced DNA damage in tumor cells. Furthermore, avoidance of apoptosis—often by TP53 mutation or overexpression of anti-apoptotic factors—allows malignant cells to sidestep cell death checkpoint impediments. Autophagy is another survival

pathway often hijacked by osteosarcoma cells to withstand chemotherapeutic insult. DNA methylation and epigenetic modifications can modulate gene expression to support chemoresistant phenotypes further, while cancer stem cells (CSCs) with high self-renewal capacity and extensive survival pathways add an additional layer to this complexity. Collectively, these interwound pathways reinforce the value of combining therapies to target multiple pathways to obtain superior efficacy and better outcomes in osteosarcoma.

2.1.1. Drug Efflux Transporters

Drug efflux transporters, particularly ATP-binding cassette (ABC) transporters, indeed play a critical role in multidrug resistance (MDR) in cancer, including osteosarcoma (OS), by actively effluxing chemotherapeutic agents from cells. The overexpression of the membrane drug transporter ATP-binding cassette subfamily B member 1 (ABCB1), also known as P-glycoprotein (P-gp) or multidrug resistance protein 1 (MDR1), has been extensively investigated in relation to chemotherapy resistance in osteosarcoma, as reported in various studies. ABCB1 plays a crucial role in OS chemoresistance to doxorubicin by actively transporting the drug out of osteosarcoma cells, thus reducing its cytotoxic effects. High ABCB1 expression combined with low ABCA1 levels is indicative of resistance to chemotherapy in OS. ABCB1 or P-gp expression is regulated by various pathways, including PI3K/Akt, PTN/β-catenin, and ERRα. These pathways influence P-gp expression through transcriptional and post-transcriptional mechanisms, such as miRNA regulation.

For instance, the miR-198/ABCB1 axis is implicated in doxorubicin resistance, where the circular RNA circ_0002060 enhances resistance by sponging miR-198, leading to increased ABCB1 expression. Furthermore, Pleiotrophin (PTN) enhances the expression of ABCB1 by activating the ALK/GSK3β/β-catenin signaling pathway, which contributes to doxorubicin resistance in OS cells. Inhibition of P-gp or its regulatory pathways can sensitize cancer cells to chemotherapy. For instance, luteolin enhances chemosensitivity in osteosarcoma by targeting the PTN/β-catenin/MDR1 axis via miR-384 upregulation. Furthermore, Inhibition of Akt, along with GRP78 suppression, has been demonstrated to decrease P-gp levels and mitigate chemoresistance in OS cells. Additionally, the tumor suppressor PTEN negatively regulates PI3K/Akt signaling, and its loss contributes to chemoresistance. Understanding these regulatory mechanisms is crucial for developing strategies to overcome drug resistance in cancer treatment.

2.1.2. Altered Drug Metabolism

One significant mechanism contributing to the chemoresistance of osteosarcoma (OS) is altered drug metabolism, which involves various metabolic pathways and cellular processes. Cytochrome P450 (CYP) enzymes and glutathione S-transferases (GSTs) play crucial roles in the development of chemoresistance in OS through altered drug metabolism. CYP, particularly CYP1A2, CYP3A4, and CYP3A5, influence the pharmacokinetics of OS chemotherapeutics, with doxorubicin upregulating CYP1A2 and CYP3A4, while cisplatin and methotrexate enhance CYP3A4. Higher expression of CYP1A2 correlates with better event-free survival, while elevated CYP3A4/5 is linked to distant metastasis and poor prognosis, indicating their potential as biomarkers. GTPs, particularly glutathione S-transferase P1 (GSTP1), expression increases in response to doxorubicin and cisplatin treatment of OS. Furthermore, GSTP1 contributes to doxorubicin and cisplatin resistance in

OS, potentially through the activation of extracellular signal-regulated kinase (ERK)1/2. Genetic variations in GSTP1 affect treatment outcomes, with the G/G genotype associated with shorter survival and poorer responses, though links with GSTM1 and GSTT1 polymorphisms are inconsistent.

The potential of CYP and GST enzyme inhibitors to enhance chemotherapy efficacy in osteosarcoma is promising due to their roles in drug metabolism and chemoresistance. Researchers are investigating multifunctional drug complexes that integrate chemotherapeutic agents with GST inhibitors. For example, platinum (IV) complexes containing GST inhibitors have been developed to address cisplatin resistance in osteosarcoma treatment. Furthermore, NBDHEX (6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol) and its analogs are promising GST inhibitors, mainly targeting GSTP1. They activate the JNK/c-Jun signaling pathway, dissociating the TRAF2-GSTP1 complex. It has been proposed as a potential treatment for cisplatin-resistant human osteosarcoma. Additionally, CYP3A4 inhibitors such as ketoconazole may help overcome chemoresistance in OS by enhancing the efficacy of chemotherapy. Although direct studies in OS are limited, findings from other cancers suggest potential benefits. For instance, the pregnane X receptor (PXR) is involved in drug metabolism and resistance in OS. Ketoconazole disrupts the interaction between PXR and HNF4α, which is essential for activating the CYP3A4 promoter. This disruption reduces CYP3A4 expression and the metabolism of chemotherapeutic agents, leading to increased intracellular concentrations and enhanced cytotoxic effects.

2.1.3. Enhanced DNA Repair Mechanisms

Chemotherapeutic drugs induce DNA damage to kill cancer cells, but tumor cells can enhance DNA repair pathways to resist treatment. The DNA damage response (DDR) plays a crucial role in maintaining genome stability and chemoresistance. Base excision repair (BER) is a crucial DNA repair mechanism that maintains genome integrity by repairing thousands of DNA lesions caused by endogenous and exogenous mutagens. In osteosarcoma (OS), BER plays a significant role in chemotherapy resistance and cancer progression. The BER pathway involves multiple steps, including base excision, strand incision, end processing, gap filling, and nick sealing, carried out by various enzymes such as DNA glycosylases, AP endonucleases, polymerases, and ligases. PARP1 (Poly (ADP-ribose) polymerase 1) plays a crucial role in the BER pathway, where it detects single-strand breaks (SSBs) in DNA and recruits repair proteins to facilitate damage repair. Knockdown of PARP1 has been found to inhibit proliferation and increase chemotherapy sensitivity to doxorubicin in osteosarcoma cells.

Several studies have identified genetic variations in the DNA repair gene, excision repair cross-complementation (ERCC), part of the nucleotide excision repair (NER) pathway, that influence the chemotherapy resistance and survival rates in osteosarcoma patients. Elevated levels of ERCC proteins are associated with resistance to cisplatin. Igarashi et al. reported that the overexpression of ERCC1 correlates with resistance to cisplatin-based chemotherapy in osteosarcoma.

The application of DNA repair inhibitors has emerged as a promising strategy to combat chemotherapy resistance, as inhibiting specific DNA repair pathways may increase

chemotherapy effectiveness. For instance, Inhibitors targeting nucleotide excision repair (NER) and base excision repair (BER) pathways and correlate with ERCC, such as NSC130813 and triptolide, have shown promise in overcoming cisplatin resistance in osteosarcoma cells. Recent studies have identified potential targets to overcome chemoresistance in OS, including PAXX, a factor in non-homologous end joining (NHEJ), a key DNA repair pathway, by using a molecule called M11 that can disrupt the PAXX-Ku70 interaction and re-sensitize the chemoresistant osteosarcoma cells to doxorubicin and cisplatin.

PARP1 is considered a potential target for overcoming chemotherapy resistance, as it enhances the repair of DNA-damaged cells by recruiting DNA damage response proteins, such as γH2AX and BRCA1/2, which are linked to reduced survival rates in osteosarcoma patients. PARP inhibitors, such as olaparib and talazoparib, have shown potential in enhancing the efficacy of chemotherapeutic agents like doxorubicin and temozolomide in OS cells, offering a potential strategy to mitigate resistance. Overall, modulating DNA damage response pathways may provide promising approaches to overcome chemoresistance in OS.

2.1.4. Apoptosis Resistance

Apoptosis resistance in osteosarcoma (OS) is one of the main obstacles in osteosarcoma treatment, mainly because of the aberrant regulation of apoptosis pathways. One of the main mechanisms of this resistance is mutations in the TP53 gene and over-expression of anti-apoptotic protein Bcl-2. TP53 mutations are common in many cancers, including osteosarcoma, causing apoptosis inhibition and malignant cell survival. In OS, the p53 R273H mutant of TP53 is correlated with reduced procaspase-3 (PC-3) expression and ineffectiveness of methotrexate and doxorubicin to induce apoptosis. The Bcl-2 family of proteins is involved in apoptosis regulation, and overexpression of anti-apoptotic proteins, such as Bcl-2 and Bcl-xL, has been associated with chemoresistance. For example, circular RNA UBAP2 (circUBAP2) was found to increase the expression of the anti-apoptotic protein Bcl-2. This suggests that circUBAP2 promotes cell survival by enhancing Bcl-2 levels, thereby preventing apoptosis in osteosarcoma cells. Furthermore, the knockdown of circUBAP2 expression inhibited the expression of Bcl-2 and significantly promotes apoptosis in osteosarcoma cells (MG63 and U2OS).

Besides genetic mutations, osteosarcoma’s intrinsic apoptotic pathway is frequently affected. The balance between pro-apoptotic proteins, such as Bax, and anti-apoptotic proteins, such as Bcl-2, primarily regulates this pathway. Overexpression of Bcl-2 has an inhibitory effect on the activation of caspases, which are necessary for the carrying out of the apoptotic program. For instance, the application of the compounds diosmetin and 6-gingerol to osteosarcoma cells has been reported to suppress Bcl-2 and augment Bax expression and, therefore, induce apoptosis. In addition, PI3K/Akt signaling pathway is frequently involved in the survival of OS cells, in which its activation can result in up-regulation of Bcl-2 and down-regulation of apoptosis. To overcome apoptosis resistance, pro-apoptotic agents and BH3 mimetics are attracting interest. These agents are designed to recover their apoptotic signaling cascades, which are frequently impaired in cancer cells. For instance, BH3 mimetics are designed to inhibit the function of anti-apoptotic Bcl-2 proteins, thereby promoting the activation of pro-apoptotic factors and facilitating cell death. Research has

demonstrated that compounds like plumbagin and honokiol can induce apoptosis in OS cells through various mechanisms, including the activation of mitochondrial pathways and the generation of reactive oxygen species (ROS). In addition, inhibition of the PI3K/Akt pathway by agents such as apatinib can increase the sensitivity of OS cells to chemotherapy by inducing apoptosis. Targeting apoptosis resistance mechanisms in osteosarcoma, such as using BH3 mimetics or disrupting mitochondrial function, may improve treatment efficacy and patient outcomes.

2.1.5. Autophagy and Cell Survival

Autophagy is an essential cellular mechanism that can degrade and recycle various cellular components and has multiple roles in cancer biology. Under the condition of chemoresistance, autophagy is known as a significant pathway through which osteosarcoma (OS) cells escape the inhibitory effects of cytotoxic drugs. Studies have shown that autophagy is upregulated in osteosarcoma cells exposed to chemotherapeutic drugs, such as doxorubicin and cisplatin, allowing these cells to survive under stress conditions. For example, autophagy induction via the Beclin-1 pathway has been associated with enhanced doxorubicin resistance, suggesting autophagy functions as a drug-resistance mechanism that prevents death by apoptosis. In addition, microRNAs (miRNAs) have been reported to regulate autophagy and chemoresistance in osteosarcoma. For instance, miR-30a downregulation has been reported to promote autophagy and thereby mediate chemoresistance. On the contrary, miR-410 has been shown to downregulate autophagy, making expression regulation of miRNA a potential sensitizing strategy for osteosarcoma cells to chemotherapy. This crosstalk between autophagy and miRNA regulation explains how autophagy contributes to cancer cell survival and drug resistance complexity.

Therapeutic implications of autophagy inhibitors, including chloroquine and related compounds, are of interest as an approach to overcoming chemoresistance in osteosarcoma. Lysosomal function inhibition by chloroquine reduces autophagic degradation and apoptosis of cancer cells. Moreover, chloroquine has been reported to block the autophagic process in cisplatin-resistant OS cells, thereby enhancing the cytotoxic effects of cisplatin. Evidence for using autophagy inhibitors in combination with existing chemotherapeutic agents has successfully improved global therapeutic efficiency. For example, inhibition of autophagy using 3-methyladenine (3-MA), an autophagy inhibitor, together with mTOR inhibitors like rapamycin has been demonstrated to promote apoptosis in OS cells, which indicates that targeting autophagy may enhance response to therapy. In contrast, 3-MA can sensitize OS cells to the mTOR inhibitor, and accordingly, autophagy has been proposed to be a master endpoint for chemoresistance. Autophagy is crucial in osteosarcoma chemoresistance, promoting cell survival during treatment. Modulating autophagy and employing inhibitors may enhance chemotherapy sensitivity, improving patient outcomes.

2.1.6. Epigenetic Modifications

Epigenetic modifications play a crucial role in developing chemotherapy resistance in osteosarcoma (OS), primarily through mechanisms involving DNA methylation and histone modifications. Aberrant DNA methylation patterns have been implicated in OS pathogenesis, particularly hypermethylation of tumor suppressor genes (TSG) and hypomethylation of

oncogenes. For instance, the overexpression of DNMT1 (DNA methyltransferase 1) has been shown to correlate with increased resistance to apoptosis in OS cells, indicating that aberrant DNA methylation patterns are a significant factor in chemoresistance. Furthermore, histone modifications, such as acetylation and methylation, are critical in regulating gene expression related to drug resistance. Histone deacetylases (HDACs) have been implicated in maintaining cancer stem cell properties and promoting resistance to chemotherapy. Specifically, the depletion of HDAC2 has been associated with enhanced stemness in OS, suggesting that histone modifications can influence the tumor's response to chemotherapy. Moreover, in OS, histone H3 lysine 27 trimethylation (H3K27me3) levels are associated with cisplatin sensitivity, with higher levels increasing chemosensitivity.

The application of epigenetic drugs, such as DNA methyltransferase and HDAC inhibitors, has emerged as a promising strategy to overcome chemotherapy resistance in OS. For example, the use of HDAC inhibitors like panobinostat has been shown to induce apoptosis and alter the expression of genes involved in drug resistance pathways, thereby enhancing the sensitivity of OS cells to chemotherapeutic agents. Additionally, combining epigenetic drugs with traditional chemotherapy has been suggested to improve treatment outcomes. Studies have indicated that inhibiting both DNA methylation and histone deacetylation can synergistically enhance the efficacy of standard chemotherapeutics like doxorubicin and cisplatin. This approach aims to reverse the epigenetic alterations contributing to drug resistance, thereby restoring osteosarcoma cells' sensitivity to chemotherapy.

Moreover, the interplay between various epigenetic modifications and non-coding RNAs, such as long non-coding RNAs (lncRNAs), further complicates the landscape of chemotherapy resistance in cancer. For instance, lncRNAs have been shown to interact with epigenetic regulators, influencing the expression of genes associated with drug resistance. The lncRNA LINC00161 has been implicated in cisplatin-induced apoptosis, where it attenuates osteosarcoma chemoresistance by targeting the miR-645-IFIT2 signaling axis. Overall, the identification of specific lncRNA signatures that correlate with treatment response could provide valuable biomarkers for predicting chemotherapy outcomes in OS patients.

2.1.7. Cancer Stem Cells

Cancer stem cells (CSCs) are essential in initiating and progressing osteosarcoma (OS) and significantly contribute to chemotherapy resistance. These cells are characterized by the overexpression of specific markers, including OCT4, SOX2, and NANOG, which are associated with stemness and drug resistance mechanism. For instance, Cavalcanti et al. highlight that patient-derived OS cells exhibit resistance to methotrexate due to the presence of CSCs that express drug transporters like ABCG2. Similarly, Liu et al. state that the intrinsic properties of CSCs are implicated in the treatment failure of OS, underscoring their role in limiting the effectiveness of conventional chemotherapy. The mechanisms of CSC-mediated drug resistance in osteosarcomas are complex and involve a wide variety of different mechanisms. Wang et al. demonstrated that the EID3 protein enhances the stem-like characteristics of osteosarcoma cells through the activation of the PI3K-AKT signaling pathway, which is known to promote cell survival and chemoresistance. Furthermore, the interplay between various signaling pathways such as Notch, Wnt/β-Catenin, and Hedgehog has been implicated in regulating the self-renewal and

survival of CSCs in OS. Targeting CSCs presents a promising non-invasive therapeutic approach to improve chemotherapy resistance in osteosarcoma. Numerous studies have reported that targeting the Hedgehog signaling pathway can lead to reduced tumor growth and increased sensitivity to chemotherapeutic agents. For example, the Hedgehog pathway inhibitor, vismodegib, has been demonstrated to block the signaling that supports the survival and proliferation of stem cells. Moreover, a study showed that the Hedgehog/GLI1 signaling pathway plays a critical role in regulating cisplatin resistance in OS, suggesting that inhibiting this pathway could enhance the efficacy of existing chemotherapeutic regimens.

2.2. Current Therapeutic Approaches to Overcome Resistance

The management of osteosarcoma (OS) faces significant challenges due to chemotherapy resistance, which dramatically impacts treatment outcomes and patient survival. Current therapeutic strategies to overcome resistance in OS include combination chemotherapy regimens, dose intensification, and novel drug delivery systems. These strategies aim to enhance the efficacy of existing treatments and introduce new modalities to improve patient outcomes. The following section thoroughly explores these strategies, highlighting their potential and limitations.

2.2.1. Combination Chemotherapy Regimens

The current therapeutic landscape for osteosarcoma (OS) predominantly involves combination chemotherapy regimens, which are essential in addressing the challenges posed by drug resistance. The standard treatment protocol typically includes agents such as doxorubicin (adriamycin), methotrexate, and cisplatin, which have been shown to significantly improve survival rates when used in conjunction with surgical interventions. This approach is necessitated by the multifaceted nature of OS, which often exhibits heterogeneity and varying responses to single-agent therapies. By employing multiple agents, clinicians aim to target different pathways involved in tumor growth and survival, thereby enhancing the overall therapeutic effect and minimizing the risk of resistance.

Combination chemotherapy regimens have evolved over the years, with the MAP (methotrexate, doxorubicin, and cisplatin) and IAP (ifosfamide, doxorubicin, and cisplatin) regimens being among the most widely adopted. These regimens have demonstrated efficacy in both neoadjuvant and adjuvant settings, improving event-free survival rates in patients with localized OS. For instance, studies indicate that the MAP regimen is associated with a 5-year survival rate of approximately 60–70% in patients without metastasis at diagnosis. However, the effectiveness of these regimens can be compromised by the development of chemotherapy resistance, which remains a significant hurdle in treatment.

Clinical outcomes from various combination strategies have yielded mixed results, with some regimens showing promise in overcoming resistance. A study on OS chemotherapy highlights the success of multidrug regimens. The T12 Protocol, which includes adriamycin, bleomycin, cyclophosphamide, dactinomycin, methotrexate, and cisplatin, demonstrates significant improvements in progression-free survival (PFS) and overall survival, especially when used with ifosfamide or vincristine. The ABCDMP regimen, which includes the same drugs as the T12 Protocol, also shows notable efficacy, particularly when paired with ifosfamide. Other effective combinations include ABCDMPL (adding vincristine) and AP (adriamycin and cisplatin). The study concludes that these multidrug approaches lead to

better survival outcomes for OS patients compared to single-agent therapies. At the same time, EURAMOS-1 trial found that adding ifosfamide and etoposide to the MAP regimen for patients with ≥10% viable tumor did not improve event-free survival and increased toxicity, indicating that such additions may not benefit poorly responding OS patients.

In chemotherapy-resistant OS models, the combination of doxorubicin and cisplatin, when paired with novel agents such as olaratumab, has proven effective, particularly as it targets specific pathways involved in tumor growth. Furthermore, incorporating agents like bortezomib has been explored, as it sensitizes OS cells to doxorubicin-induced apoptosis by activating specific signaling pathways. Moreover, combining curcumol with cisplatin has demonstrated synergistic effects in preclinical models by inhibiting M2-like polarization of tumor-associated macrophages, which may contribute to chemoresistance. Additionally, the combination of docetaxel and gemcitabine has been evaluated for its efficacy in treating relapsed and refractory OS, showing improved response rates, particularly in older patients who may be more susceptible to chemotherapy-related toxicities. In a patient-derived orthotopic xenograft (PDOX) model, the combination of temozolomide and irinotecan demonstrated significant efficacy in regressing cisplatin-resistant OS, suggesting a potential treatment strategy for patients with relapsed disease. These findings underscore the potential for combination therapies to enhance the efficacy of existing chemotherapeutic agents and improve patient outcomes. They also highlight the need to carefully evaluate treatment strategies to maximize efficacy and minimize adverse effects.

2.2.2. Dose Intensification and Modification

Dose intensification and modification are crucial in managing osteosarcoma (OS), particularly in addressing the significant resistance that OS exhibits to conventional chemotherapy regimens. OS exhibits significant resistance to conventional chemotherapy regimens, necessitating the exploration of high-dose chemotherapy protocols and careful modification of treatment strategies to enhance therapeutic efficacy while managing toxicity. The standard chemotherapy regimen typically includes high-dose methotrexate (HDMTX), doxorubicin, and cisplatin, which have been shown to improve survival rates in patients with OS significantly. The EURAMOS-1 protocol highlights the importance of histologic response to neoadjuvant chemotherapy as a survival predictor, making it essential for treatment planning. Research shows that a higher number of high-dose methotrexate (HDMTX) doses is linked to improved histologic response and survival outcomes, highlighting the need for dose intensification to achieve better therapeutic results.

High-dose chemotherapy protocols, particularly those incorporating HDMTX, are pivotal in addressing the chemoresistance observed in OS. The administration of HDMTX, often in combination with agents like doxorubicin and cisplatin, has been associated with increased tumor necrosis and improved overall survival rates. However, these high-dose regimens are not without risks; they can lead to significant hematological complications such as thrombocytopenia and leukopenia, which complicate treatment and may necessitate dose modifications.

The impact of dose intensification is, thus, a double-edged sword; while higher doses can lead to better tumor control, they also increase the risk of adverse effects, potentially resulting in treatment delays or dose reductions. Consequently, balancing therapeutic

efficacy with toxicity is a critical consideration in the management of osteosarcoma. This balance is essential to optimize treatment outcomes while minimizing the risks associated with high-dose chemotherapy protocols.

2.2.3. Novel Drug Delivery Systems

Novel Drug Delivery Systems (NDDS) play a crucial role in overcoming chemotherapy resistance in osteosarcoma (OS). The challenges posed by multidrug resistance (MDR) in OS necessitate innovative strategies to enhance drug efficacy and minimize systemic toxicity. NDDS can bypass drug efflux mechanisms, control drug release, and disturb tumor metabolism to combat MDR. Among these strategies, nanoparticle-based delivery systems and liposomal formulations have emerged as promising approaches, particularly through targeted drug delivery mechanisms.

Nanoparticle-based delivery systems have shown significant potential in addressing the limitations of conventional chemotherapy. These systems can improve drug solubility, enhance drug accumulation at tumor sites, and reduce systemic side effects through the enhanced permeability and retention (EPR) effect. For instance, studies have demonstrated that nanoparticles can effectively deliver chemotherapeutic agents like doxorubicin directly to OS cells, thereby increasing the local concentration of the drug while reducing side effects. Various nanocarriers, including polypeptide nanoparticles, biomimetic nanoparticles, and liposomes, have been developed to improve drug delivery. These nanocarriers can protect drugs from rapid clearance, prolong circulation time, and increase drug concentration at tumor sites. Some nanoparticles are designed to respond to specific stimuli, such as pH or reactive oxygen species, enabling controlled drug release. Furthermore, the use of hybrid nanoparticles, such as lipid–polymer hybrids, has been reported to synergistically enhance the anticancer effects of drugs like doxorubicin and edelfosine against drug-resistant osteosarcoma.

Liposomal formulations represent a significant advancement in the targeted delivery of chemotherapeutics for osteosarcoma treatment. These lipid-based carriers can encapsulate drugs, protecting them from degradation and facilitating controlled release. Several liposomal formulations have been developed and tested in clinical settings for osteosarcoma treatment. For example, Doxil (liposomal doxorubicin) has shown improved efficacy and reduced cardiotoxicity compared to free doxorubicin in various cancers, including osteosarcoma. Liposomes can be engineered to target specific receptors overexpressed in OS cells, such as CD44, which is associated with drug resistance. By modifying liposomal formulations to include targeting ligands, researchers have been able to enhance the uptake of chemotherapeutic agents by OS cells, thereby overcoming some of the barriers posed by MDR mechanisms.

Targeted drug delivery mechanisms are pivotal in enhancing the therapeutic efficacy of NDDS in osteosarcoma treatment. These mechanisms can be classified into passive and active targeting. Passive targeting relies on the enhanced permeability and retention (EPR) effect, where nanoparticles accumulate in tumor tissues due to their leaky vasculature. Active targeting, on the other hand, involves the functionalization of nanoparticles with specific ligands that bind to receptors on the surface of cancer cells, facilitating increased internalization of the drug. For example, the use of folate receptor-targeted nanoparticles

has been shown to significantly improve the delivery of doxorubicin to OS cells, leading to enhanced cytotoxicity and reduced drug resistance. Furthermore, nanocarriers, such as micelleplexes, have been developed for the active targeting of OS cells to overcome multidrug resistance (MDR) and non-specific toxicity by incorporating targeting ligands that selectively bind to OS cell receptors, thereby enhancing drug delivery efficacy and reducing systemic toxicity. Additionally, targeting the Ras/Akt/mTOR and Ras/ERK1/2/HIF-1α pathways with self-assembling nanoparticles encapsulating zoledronic acid (NZ) can simultaneously upregulate ABCA1 and downregulate ABCB1, thereby restoring drug sensitivity in doxorubicin-resistant osteosarcoma. The integration of novel drug delivery systems, particularly nanoparticle-based and liposomal formulations, into osteosarcoma treatment regimens, holds great promise for overcoming chemotherapy resistance. By leveraging targeted drug delivery mechanisms, these systems can enhance drug accumulation at tumor sites, improve therapeutic outcomes, and potentially reduce the incidence of systemic side effects associated with traditional chemotherapy.

3. Targeted Therapies Based on Molecular Abnormalities

3.1. Genetic and Molecular Targets in Osteosarcoma

The molecular landscape of osteosarcoma is complex, with receptor tyrosine kinases (RTKs) such as IGF-1R, PDGFR, and HER2 playing pivotal roles in tumor growth and progression. These RTKs are often overexpressed in osteosarcoma, making them critical targets for therapeutic intervention.

3.1.1. Receptor Tyrosine Kinases (RTKs)

The insulin-like growth factor 1 receptor (IGF-1R) is frequently overexpressed in osteosarcoma, contributing to enhanced cell proliferation and survival through the activation of downstream signaling pathways, including the PI3K/Akt and MAPK pathways. This overexpression correlates with poor clinical outcomes, as it promotes tumorigenesis and resistance to conventional therapies. Targeting IGF-1R with specific inhibitors has shown promise in preclinical studies, suggesting that it may serve as a viable therapeutic target in osteosarcoma treatment. However, IGF-1R inhibitors have failed in clinical trials due to the complex interactions of IGF-1R with adhesion receptors and the tumor microenvironment, as well as the insufficient understanding of its role in tumor-associated immune and stromal cells, although some early-phase trials showed evidence of response, particularly in sarcomas. Similarly, the platelet-derived growth factor receptor (PDGFR) is implicated in osteosarcoma pathogenesis. Studies have demonstrated that PDGFRα and PDGFRβ are expressed in a significant percentage of osteosarcoma samples, and their activation is associated with increased tumor growth and metastasis. Inhibition of PDGFR signaling has been explored as a therapeutic strategy, with agents like imatinib mesylate showing anti-proliferative effects on osteosarcoma cells in vitro and in vivo. However, clinical trials have indicated limited efficacy, suggesting that alternative pathways may compensate for PDGFR inhibition. HER2, another RTK, has also been identified as an important player in osteosarcoma. Overexpression of HER2 has been linked to aggressive tumor behavior and poor prognosis. Targeting HER2 with monoclonal antibodies or small molecule inhibitors may provide a therapeutic avenue, particularly for patients with HER2-positive tumors. The

integration of HER2-targeted therapies with existing treatment modalities could enhance therapeutic efficacy and improve patient outcomes.

3.1.2. Multi-Kinase Inhibitors (MKIs)

Multi-Kinase Inhibitors (MKIs) are emerging as promising therapeutic agents in the treatment of osteosarcoma (OS) due to their ability to target multiple pathways implicated in tumor growth and resistance mechanisms. OS is characterized by complex genetic alterations and dysregulation of multiple signaling pathways, including PI3K/Akt/mTOR, JAK/STAT, Wnt/β-catenin, Hippo, Notch, PD-1/PD-L1, MAPK, and NF-κB. MKIs target multiple kinases involved in tumor growth, metastasis, and drug resistance. MKIs have shown promise in targeting various kinases in osteosarcoma treatment. These include receptor tyrosine kinases like VEGFRs and RET, which are considered key targets. Cell cycle kinases such as CHKs, CDKs, PLKs, and AURKs are also potential therapeutic targets. Specific kinases identified as crucial for osteosarcoma cell survival include Mirk and PLK1. Several MKIs have demonstrated efficacy in preclinical models and clinical trials. For example, regorafenib, a potent MKI, has shown regression in patient-derived orthotopic xenograft models and outperformed other MKIs while also demonstrating regression in drug-resistant osteosarcoma models. Furthermore, regorafenib, sorafenib, apatinib, and cabozantinib target multiple receptor tyrosine kinases, including VEGFRs and RET, which are considered key targets in osteosarcoma treatment and have shown clinical benefit in phase II trials. Sorafenib inhibited tumor growth, angiogenesis, and metastasis in preclinical models by targeting ERK1/2, MCL-1, and ERM pathways. Combining sorafenib with everolimus enhanced its antitumor activity by completely inhibiting the mTOR pathway. Cabozantinib inhibits OS cell proliferation and migration while also modifying the bone microenvironment. While these MKIs show promise, larger-scale trials are needed to validate their efficacy and identify predictive biomarkers for response. Furthermore, MKIs such as anlotinib, apatinib, and sorafenib have improved progression-free survival in advanced osteosarcoma; however, overall survival remains unchanged due to the rapid development of acquired drug resistance in osteosarcoma.

3.1.3. Signal Transduction Pathways

The PI3K/Akt/mTOR signaling pathway plays a critical role in the pathogenesis and progression of osteosarcoma. Dysregulation of this pathway is frequently observed in osteosarcoma, leading to enhanced cell proliferation, survival, and metastasis. The activation of PI3K leads to the phosphorylation of Akt, which subsequently activates mTOR, a central regulator of cell growth and metabolism. This cascade of events contributes to the aggressive nature of osteosarcoma by promoting tumor cell proliferation and inhibiting apoptosis. Studies have shown that the aberrant activation of the PI3K/Akt/mTOR pathway is a pivotal event in osteosarcoma development. For instance, Jin et al. demonstrated that silencing GPNMB, a glycoprotein associated with tumor progression, suppressed osteosarcoma cell proliferation and metastasis by inhibiting this pathway. Similarly, Zheng et al. reported that AIM2, a DNA-binding protein, could inhibit osteosarcoma cell proliferation and promote apoptosis by inactivating the PI3K/Akt/mTOR signaling pathway. Furthermore, the involvement of this pathway in epithelial–mesenchymal transition (EMT) has been highlighted, indicating its role in enhancing the invasive potential of osteosarcoma cells.

Therapeutic agents targeting mTOR have emerged as promising strategies for treating osteosarcoma.

mTOR inhibitors, such as rapamycin and its analogs (e.g., everolimus), have shown potential in preclinical studies and clinical trials. For instance, Gupte et al. identified dual inhibition of PI3K and mTOR as a conserved therapeutic vulnerability in osteosarcoma, suggesting that targeting this pathway could enhance treatment efficacy. Moreover, the combination of mTOR inhibitors with other agents, such as zoledronic acid, has been shown to overcome resistance to conventional therapies, further supporting the therapeutic potential of mTOR inhibition. In addition to mTOR inhibitors, other therapeutic agents targeting the PI3K/Akt/mTOR pathway have been investigated. For example, the dual PI3K/mTOR inhibitor NVP-BEZ235 was found to inhibit osteosarcoma cell proliferation and improve survival rates in vivo. Other studies have explored the effects of natural compounds, such as honokiol and alantolactone, which induce apoptosis and autophagy in osteosarcoma cells through the inhibition of the PI3K/Akt/mTOR pathway. These findings underscore the importance of targeting the PI3K/Akt/mTOR signaling pathway as a viable therapeutic strategy in osteosarcoma management.

3.1.4. MicroRNA-Based Therapies

MicroRNA (miRNA)-based therapies have emerged as a promising avenue for the modulation of gene expression in treating osteosarcoma. miRNAs are small, non-coding RNA molecules that play critical roles in regulating gene expression by binding to the 3′ untranslated regions (UTRs) of target mRNAs, leading to their degradation or translational repression. This regulatory function is particularly relevant in cancer, where the dysregulation of miRNAs can contribute to tumorigenesis, metastasis, and resistance to therapies. In osteosarcoma, several studies have identified specific miRNAs that are either upregulated or downregulated, influencing tumor behavior and patient prognosis. For instance, miR-21 has been shown to promote cell invasion and migration by targeting the tumor suppressor gene RECK, thereby enhancing the metastatic potential of osteosarcoma cells. Conversely, miR-140-5p has been identified as a tumor suppressor that inhibits cancer cell proliferation by downregulating GLUT-1, suggesting that restoring its expression could be beneficial in osteosarcoma treatment.

Therapeutic delivery systems for miRNAs are crucial for their practical application in clinical settings. Nanoparticle-based delivery systems, such as dendritic polyglycerol nanopolyplexes, have been developed to enhance the stability and bioavailability of miRNAs in vivo. These systems can facilitate the targeted delivery of miRNAs to tumor cells, thereby improving therapeutic outcomes. For example, the use of miR-200 family members has been explored for their ability to act as tumor suppressors by targeting genes involved in epithelial-to-mesenchymal transition (EMT), which is a critical process in cancer metastasis. Moreover, the identification of circulating exosomal miRNAs in osteosarcoma has opened new avenues for non-invasive diagnostics and monitoring of treatment response. The potential of miRNAs as biomarkers for osteosarcoma is underscored by studies showing that specific miRNAs correlate with disease progression and patient survival. For instance, miR-26a downregulation has been associated with poor prognosis in osteosarcoma patients, indicating its role in tumor metastasis.