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Targeting Protein Kinases to Reverse Multidrug Resistance in Sarcoma

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Abstract

Sarcomas are a group of cancers that arise from transformed cells of mesenchymal origin. They can be classified into over 50 subtypes, accounting for approximately 1% of adult and 15% of pediatric cancers. Wide surgical resection, radiotherapy, and chemotherapy are the most common treatments for the majority of sarcomas. Among these therapies, chemotherapy can palliate symptoms and prolong life for some sarcoma patients. However, sarcoma cells can have intrinsic or acquired resistance after treatment with chemotherapeutic drugs, leading to the development of multidrug resistance (MDR). MDR attenuates the efficacy of anticancer drugs and results in treatment failure for sarcomas. Therefore, overcoming MDR is an unmet need for sarcoma therapy. Certain protein kinases demonstrate aberrant expression and/or activity in sarcoma cells, which have been found to be involved in the regulation of sarcoma cell progression, such as cell cycle, apoptosis, and survival. Inhibiting these protein kinases may not only decrease the proliferation and growth of sarcoma cells, but also reverse their resistance to chemotherapeutic drugs to subsequently reduce the doses of anticancer drugs and decrease drug side-effects. The discovery of novel strategies targeting protein kinases opens a door to a new area of sarcoma research and provides insight into the mechanisms of MDR in chemotherapy. This review will focus on the recent studies in targeting protein kinase to reverse chemotherapeutic drug resistance in sarcoma.

Keywords

multidrug resistance (MDR); protein kinase; sarcoma

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Introduction

Sarcomas are a heterogeneous group of malignant tumors that arise from transformed cells of mesenchymal origin. Sarcomas are divided into two main groups: soft tissue sarcomas and bone sarcomas. Soft tissue sarcomas (STS) originate in connective tissues such as fat, muscle, nerve, tendon, the lining of joints, blood vessels, or lymph vessels. There are more than 50 different histological subtypes of STS, such as undifferentiated pleomorphic sarcoma (UPS), rhabdomyosarcoma, liposarcoma, angiosarcoma, synovial sarcoma, leiomyosarcoma, and others. Bone sarcomas develop from bone, and include osteosarcoma, Ewing sarcoma, and chondrosarcoma. There are 15,000 new sarcoma cases per year in the United States, consisting of 12,000 cases of STS and 3,000 cases of bone sarcomas¹. The 5-year overall survival rate is approximately 50–80% for sarcomas².

Surgical resection, radiotherapy, and systemic chemotherapy comprise the standard treatments for sarcoma. The application of multi-agent chemotherapy and appropriate surgical resection has significantly improved the survival rate and quality of life after treatment for patients with certain types of sarcoma, including osteosarcoma, rhabdomyosarcoma, and Ewing sarcoma. For example, major progress has been made in the treatment of patients with osteosarcoma because of the use of chemotherapy, leading to an improved overall survival rate of up to 65%. Chemotherapy drugs usually include doxorubicin, methotrexate, ifosfamide, and cisplatin. Unfortunately, the efficacy of these agents is often hampered by the development of multidrug resistance (MDR). In osteosarcoma, 30% to 40% of patients will experience MDR associated with recurrence or metastasis despite improved multimodality therapy³. A number of patients will also develop resistance to multiple types of chemotherapy after prolonged periods of treatment. Drug resistance to various functionally and structurally unrelated chemotherapeutic drugs in sarcoma cells may be intrinsic or acquired, and subsequently limits the overall utility of chemotherapy. Improvements to the survival rate of sarcoma patients have reached a plateau in the past few decades. Almost one third of patients with localized sarcoma experience recurrent or progressive disease, and the average survival period after recurrence is about one year. The mechanism of MDR in sarcoma is not well understood. There is a wide range of mechanisms that contribute to drug sensitivity/resistance, including ATP-binding cassette transporter (ABC transporters) mediated drug efflux, alteration of apoptosis, cancer stem cells (CSC), alteration/mutation of specific targets of the drugs, aberrant activation cell signaling pathways, DNA damage and repair, autophagy induction, miRNA regulation, hypoxia induction, epigenetic regulation, tumor microenvironment, etc. Overexpression/activation of protein kinases in sarcoma has also been recently identified, which enables sarcoma cells to escape the cytotoxic effects of chemotherapeutic agents. Strategies to reverse MDR have been a high priority goal for clinical and investigational sarcoma oncologists. One promising approach is the specific targeting of protein kinases implicated in different types of sarcoma to reverse MDR⁴.

The human kinome contains at least 600 protein kinases that carry out the phosphorylation of proteins at 250,000 or more sites. Generally, protein kinases are divided into tyrosine, serine/threonine, histidine, and mixed protein kinases based on their phosphorylated substrates. Protein kinases are vital to regulate many tumor processes, including cell growth,

survival, angiogenesis, apoptosis, recurrence, and metastasis. Consequently, protein kinases have emerged as one of the most promising therapeutic target families for the treatment of cancers. To date, the Food and Drug Administration (FDA) has approved approximately 30 protein kinases inhibitors for clinical use⁵. Though the understanding of the functional roles in the kinome for MDR is not yet understood, studies of these protein kinases and their functions will contribute to the discovery and development of new therapeutic strategies. A number of protein kinases have been found to be highly expressed and activated in a variety of sarcomas, particularly in the late stage of drug resistant tumors⁶. Suppression of several protein kinases, such as mammalian target of rapamycin (mTOR), SRC non-receptor tyrosine kinase (SRC), insulin-like growth factor 1 (IGF-1) receptor (IGF-1R), epidermal growth factor receptor (EGFR), c-Jun N-terminal kinases (JNKs), Janus kinase (JAK), mitogen-activated protein kinase (MAPK), or extracellular signal-regulated kinase (ERK), enhances cell death in the presence of low concentrations of chemotherapeutic drug, implicating the potential utility of these protein kinases as drug targets⁷.

Here, we present an overview of the most recent targets within the protein kinases, which may reverse chemotherapeutic drugs resistance in sarcomas.

Targeting tyrosine kinases to reverse MDR in sarcomas

Tyrosine kinases (TKs) catalyze the transfer of a phosphate of ATP to tyrosine residues on protein substrates. Tyrosine kinases play a key role in signal transduction and regulating cell cycle, mitogenesis, proliferation, differentiation, adhesion, migration, and apoptosis⁸. The tyrosine kinase families include 90 tyrosine kinases in the human genome and are divided into two main subfamilies: receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). RTKs consist of an extracellular domain, a transmembrane domain, and an intracellular catalytic domain. A specific ligand (e.g., a particular growth factor or hormone) binds the extracellular domain and triggers a cascade of reactions; as a result, an extracellular signal is transduced to the nucleus through phosphorylation of intracellular substrates proteins, which consequently regulates the expression of gene and protein function. There are 58 types of RTKs, distributed into 20 subfamilies, including EGFR, vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), anaplastic lymphoma receptor tyrosine kinase (ALK), AXL receptor tyrosine kinase (AXL), MET proto-oncogene (MET, also known as Hepatocyte Growth Factor Receptor), and insulin receptor (INSR). NRTKs are phosphotransferase enzymes that are responsible for catalyzing the transfer of a phosphate group from ATP to tyrosine residues in proteins. NRTKs transfer extracellular signals to the nucleus, regulate cellular diverse processes, and are also vital to regulating the immune system. Unlike RTKs, most NRTKs are localized in the cytoplasm; however, there are few NRTKs anchored to the cell membrane through amino-terminal modification, whereas others lack an extracellular ligand-binding domain and transmembrane-spanning region. Thirty-two types of NRTKs have been identified and divided into 10 subfamilies, including SRC, ABL, JAK, and Focal adhesion kinase (FAK). Aberrant expression of certain tyrosine kinases and/or constitutive activation of downstream pathways may be responsible for the progression of sarcoma, including tumor cell survival, apoptosis, neovascularization, and invasion, particularly in MDR⁹. Accumulating evidence reveals that

tyrosine kinases are highly expressed in sarcoma, such as overexpression of JAK1 in Ewing sarcoma, EGFR and PDGFRA in synovial sarcoma, and FGFR in osteosarcoma and rhabdomyosarcoma¹⁰. Targeting tyrosine kinases can not only lead to inhibition of these kinases and downregulation of downstream signal pathways to decrease tumor cell growth, but may also present a potential strategy to reverse MDR in sarcoma (Figure. 1, Table 1).

Targeting receptor tyrosine kinases (RTKs)

Targeting EGFR (epidermal growth factor receptor)

EGFR is a receptor tyrosine kinase of the ErbB family, which also includes ErbB2 (human epidermal growth factor receptor-2 (HER2)), ErbB3 (HER3), and ErbB4 (HER4). EGFR may form an activated heterodimer conjugate with another member of the ErbB receptor family, such as ErbB2. EGFR dimerization and the subsequent autophosphorylation of tyrosines induces various downstream signal transduction cascades and lysosomal degradation. The downstream signals predominantly activate the Ras/Raf/MEK/ERK, PI3K/AKT, JNK, signal transducer and activator of transcription (STAT), and PLC γ /PKC pathways, leading to DNA synthesis and regulation cell proliferation, adhesion, and differentiation¹¹. EGFR is both overexpressed and activated in various sarcomas, including osteosarcoma, fibrosarcoma, rhabdomyosarcoma, and liposarcoma, which promotes tumor cell progression, activation of downstream signal transduction pathways, resistance to chemotherapeutic drugs, and is associated with poor prognosis¹². Targeting EGFR with small molecule kinase inhibitors and monoclonal antibodies (mAb) has become a rational targeting strategy for the treatment of sarcoma. Recent studies have demonstrated *in vitro* and *in vivo* efficacy of the small molecule kinase inhibitor imatinib to treat osteosarcoma and the mAb cetuximab for the treatment of rhabdomyosarcoma^{13, 14}.

In a study that demonstrated highly activated EGFR in leiomyosarcoma, the use of an EGFR inhibitor showed a potent ability to overcome MDR⁹. The group investigated the effect of EGFR inhibitor gefitinib on leiomyosarcoma stem-like cells. It is believed that stem-like cells specifically use their stem cell processes of self-renewal and asymmetric differentiation to develop into various cancer cell types and are characterized by their innate MDR to chemotherapy¹⁵. These leiomyosarcoma stem-like cells exhibit chemoresistance and show high activation of AKT and ERK pathways. Treatment with EGFR inhibitor gefitinib reduced PI3/AKT and MAPK/ERK pathway activation and chemosensitized leiomyosarcoma stem-like cells *in vitro*. Moreover, gefitinib used as single agent killed tumor cells *in vitro*, reduced tumor growth *in vivo*, and in combination with vincristine, showed additive effects by increasing the activity of vincristine. Furthermore, gefitinib decreased anti-apoptotic factor Bcl-2 levels and increased intracellular doxorubicin retention. The combination of gefitinib with gemcitabine also enhanced inhibition of tumor cell proliferation, increased tumor cell apoptosis, and induced G1 cell cycle arrest *in vitro*. *In vivo*, the combination of gefitinib and doxorubicin resulted in a markedly synergistic effect on tumor cell growth inhibition and apoptosis. In addition to STS such as leiomyosarcoma as described above, EGFR is also overexpressed and highly activated in chondrosarcoma cell lines. Accordingly, overexpression of EGFR contributes to cisplatin resistance in chondrosarcoma¹⁶. The combination of erlotinib (an inhibitor of EGFR that specifically

targets the ATP binding site of the receptor in a reversible fashion) with cisplatin showed a sensitization effect in chemoresistant chondrosarcoma cells that induced apoptosis and cell cycle arrest¹⁶.

An anti-EGFR mAb is designed to specifically bind to the extracellular domain of EGFR, which creates a competitive ligand inhibition, and subsequently prevents receptor dimerization, inhibits receptor phosphorylation, and suppresses downstream signal pathways. Cetuximab, a recombinant chimeric human murine IgG1 type mAb, is the first anti-EGFR mAb approved by the FDA for clinical use. Cetuximab increases lysis in EGFR-expressing chemoresistant osteosarcoma cell lines by enhancing the cytotoxic function of resting natural killer (NK) cells¹⁷. Consequently, cetuximab-mediated anti-EGFR effects related to immunotherapy should be a novel subject for further research studies in the management of chemoresistant sarcomas. Collectively, targeting EGFR represents a promising therapeutic strategy for overcoming MDR in sarcoma.

Targeting AXL receptor tyrosine kinase

AXL (also known as ARK; UFO; JTK11; Tyro7) is a member of the receptor tyrosine kinase TAM subfamily, which also includes Tyro3 and Mer. AXL is ubiquitously expressed in epithelial cells, endothelial cells, mesenchymal, and hematopoietic origins throughout all tissues. Overexpression and activation of AXL was found to be correlated with poor survival in a variety of sarcomas, including osteosarcoma, liposarcoma, rhabdomyosarcoma, and leiomyosarcoma^{18–21}. AXL plays a pivotal role in tumor cell differentiation, apoptosis, invasion, and metastasis through PI3K/AKT/S6K, MAPK/ERK, IL-6-STAT-3, NF- κ B pathways, and the epithelial-to-mesenchymal transition (EMT) phenotype²². Targeting the 3'-UTR of AXL with miR-199a-3p downregulated the expression of the AXL gene and subsequently inhibited the progression in osteosarcoma cells through the AKT pathway²³.

Recently, a study demonstrated that there was abundant expression of AXL and Gas6 in Ewing sarcoma patient samples²⁴. Higher levels of AXL correlated with a worse prognosis compared with lower expression in primary patient tissues. *In vitro*, BGB324, an AXL kinase inhibitor, decreased viability and migration in Ewing sarcoma cell lines. More importantly, the Ewing sarcoma cell lines most resistant to vincristine and doxorubicin are associated with higher AXL and Gas6 expression levels. Furthermore, treatment of BGB324 sensitized chemoresistant cell lines to vincristine and doxorubicin. The study demonstrated that treatment of BGB324 reduced expression of pAKT and pERK levels and reversed MDR in Ewing sarcoma cells; however, the latter may be related to downregulation of PI3K/AKT and ERK pathways, which need further investigation²⁵. From a clinical perspective, the combination of BGB324 with chemotherapeutic drugs may benefit Ewing sarcoma patients with chemoresistance.

In addition to EGFR and AXL as described above, there are several other RTKs that have also been identified to be involved in MDR sarcomas, such as IGF-1R, PDGFR, VEGFR, and MET.

Targeting other RTKs

The insulin-like growth factor 1 receptor (IGF-1R) binds IGF-1 and IGF-2, and subsequently activates two main downstream signaling pathways, Ras/Raf/MEK/ERK and PI3K/AKT pathways, which are implicated in tumor cell proliferation, survival, growth, neovascularization, migration, invasion, and metastasis. Expression of IGF-1R has been found in various sarcomas, including rhabdomyosarcoma, osteosarcoma, synovial sarcoma, leiomyosarcoma, and Ewing sarcoma, and represents a negative prognostic biomarker for patients^{26–31}. AVE1642, a mAb directed against IGF-1R, showed synergistic effects in the treatment of leiomyosarcoma in combination with gemcitabine³¹. Targeting IGF-1R with AG1024, a commercially available small molecule kinase inhibitor, showed enhanced doxorubicin chemosensitivity in human osteosarcoma cell lines³². Targeting IGF-1R via RNAi also showed a marked chemosensitization effect in osteosarcoma cells³³. In addition, a recent study demonstrated that chimaphilin, a naphthoquinone extracted from pyrola, could induce apoptosis and reverse MDR osteosarcoma cell lines by targeting IGF-1R signaling³⁴.

The PDGFR includes two types of receptors: alpha-type and beta-type PDGFRs. When the receptor binds its ligands (PDGF-AA, -BB, -AB, -CC, -DD), downstream signal transduction pathways become activated, such as PI3K and STAT3 pathways. Overexpression and constitutive activation of PDGFR play a key role in regulation of cell growth, differentiation, proliferation, and migration, particularly in blood vessel formation (angiogenesis) in sarcomas³⁵. Notably, in a multi-platform profiling study of 2539 sarcoma specimens covering 22 sarcoma subtypes, researchers demonstrated that PDGFRA was overexpressed in 22.1% of these sarcomas, including in angiosarcoma, liposarcoma, fibrosarcoma, Ewing sarcoma, chondrosarcoma, osteosarcoma, UPS, and leiomyosarcoma³⁶. Olaratumab, a human monoclonal antibody that directly binds PDGFRA and blocks ligand binding, concurrent with doxorubicin, dramatically improved overall survival in advanced/metastatic STS in a randomized phase Ib/II trial; a phase III trial is currently underway³⁷. Targeting PDGFR with the small molecule inhibitor imatinib exhibits a synergistic anti-proliferative effect on osteosarcoma when combined with doxorubicin³⁸. Sorafenib is a small molecule multi-target kinase inhibitor of several protein kinases, including VEGFR, PDGFR, and Raf kinases, that showed a synergistic effect on osteosarcoma and Ewing sarcoma in combination with topotecan⁴.

VEGFR is vital in vasculogenesis and angiogenesis, and represents an attractive strategy to block the development of new blood supply for tumors by targeting VEGFR³⁹. Overexpression and/or aberrant activation of VEGFR are associated with poor prognosis in sarcomas⁴⁰. Recently, a phase II clinical trial demonstrated that bevacizumab, a humanized monoclonal antibody that targets VEGF-A, inhibited the binding of VEGFR and showed favorable activity in combination with chemotherapeutic drugs in STS⁴¹. In another multicentre, non-randomized phase II study conducted by the Italian Sarcoma Group, researchers demonstrated that the combination of sorafenib with the mTOR inhibitor everolimus showed favorable 6-month progression-free survival for 45% of patients with relapsed or unresectable high-grade osteosarcoma, who had progressed after failure of the standard chemotherapeutic drugs treatments⁴².

MET, also called c-Met and hepatocyte growth factor receptor (HGFR), is a membrane receptor tyrosine kinase encoded by the MET oncogene, which binds HGF and subsequently transduces signal through MAPK, PI3K, STAT, Notch, and Wnt signaling pathways. Overexpression and aberrantly activated MET triggers cell growth, angiogenesis, invasion, and metastasis correlated with poor prognosis in various sarcomas^{43, 44}. Targeting MET using the small molecule kinase inhibitor PHA-665752 and the neutralizing anti-HGF antibody sensitized osteosarcoma cells to cisplatin through suppression of the PI3K/AKT signaling⁴⁵. PHA-665752 and neutralizing anti-HGF antibody substantially decreased the levels of pAKT with no changes on total AKT levels.

Targeting non-receptor tyrosine kinases (NRTKs)

Targeting SRC Kinase

The SRC family kinases (SFKs) have nine members, including SRC, Lck, Yes, Fyn, Fgr, Yrk, Lyn, Blk, and Hck. In humans, SRC, Fyn, and Yes are ubiquitously expressed in all tissues, while the others are generally expressed in hematopoietic cells, with high levels in platelets, neural tissue, and osteoclasts with SRC. SRC can be activated by many different proteins, including RTKs, adhesion receptors, integrins, G-protein coupled receptors, cytokine receptors, as well as immune response receptors. The SRC kinase plays a crucial role in promoting tumor cell proliferation, survival, adhesion, migration, invasion, angiogenesis, and reducing apoptosis by interacting with other tyrosine kinase receptors and intracellular signaling pathways, such as PDGFR, EGFR, VEGFR, PI3K/AKT, and Ras/Raf/MEK/MAPK signal pathways⁴⁶.

There is increasing evidence that demonstrates that SFKs are expressed and hyperactivated in most sarcomas, including in osteosarcoma, Ewing sarcoma, liposarcoma, leiomyosarcoma, synovial sarcoma, fibrosarcoma, and rhabdomyosarcoma^{47–53}. Therefore, targeting SFKs represents a promising therapeutic target. Several small molecule kinase inhibitors of SFKs have demonstrated efficacy in sarcoma cells, such as SI-83 in osteosarcoma; AP23994 in Ewing sarcoma; dasatinib in osteosarcoma, Ewing sarcoma, liposarcoma and synovial sarcoma; SI221 in rhabdomyosarcoma; and saracatinib in fibrosarcoma^{54, 55}.

A-770041 is a potent SFK (Lck and SRC) inhibitor, which not only showed inhibition of SRC, but also reversed MDR ability in osteosarcoma⁵⁶. The combination of A-770041 with paclitaxel and doxorubicin sensitized and reversed chemotherapeutic drugs resistance in MDR human osteosarcoma cell lines. Moreover, lentiviral shRNA targeting SRC induced downregulation of SRC expression levels and reversed MDR. A-770041 significantly increased intracellular accumulation of drug concentration in MDR osteosarcoma cell lines that overexpressed ABCB1 (also known as P-gp, MDR1) in a dose-dependent manner without altering the expression of ABCB1 protein levels. These results indicated that A-770041 inhibited ABCB1 efflux function via interaction with ABCB1 and contributed to the reversal of MDR. In addition, A-770041 in combination with doxorubicin increased apoptosis to further enhance chemosensitization in MDR osteosarcoma cell lines. Dasatinib also showed a potent sensitization effect with doxorubicin treatment in TP53 mutant chemoresistant chondrosarcoma cells exhibited by increased doxorubicin induced-

apoptosis⁵⁷. In synovial sarcoma, dasatinib inhibited tumor cell growth and induced apoptosis *in vitro* and *in vivo*, leading to decreased phosphorylation of FAK, STAT3, IGF-IR, and AKT. Concurrent administration of dasatinib with chemotherapeutic agents also displayed additive effects in synovial sarcoma⁵⁰.

Targeting other non-receptor tyrosine kinases (NRTKs)

Nilotinib, a small molecule multi-target tyrosine kinase inhibitor, inhibits the BCR-ABL, c-KIT, PDGFRB, and MAPK11 kinases. Recently, a study demonstrated that nilotinib plays an important role in inhibiting tumor cell growth and overcoming MDR in STS⁷. The group showed that the combination of nilotinib with doxorubicin exhibited synergistic antitumor effects and apoptosis in synovial sarcoma and leiomyosarcoma cells. Moreover, nilotinib inhibited ABCB1 and ABCC1 (also known as MRP1) activity, which resulted in increased intracellular doxorubicin accumulation. Furthermore, nilotinib decreased basal ABCB1 expression levels and p38 MAPK phosphorylation, as well as fully inhibited overexpression of ABCB1 levels and p38 MAPK phosphorylation induced by doxorubicin in synovial sarcoma cell lines. In addition, the study demonstrated that p38 MAPK activation also results in the development of MDR by modulating the expression and activity of ABCB1. Given the ABC transporters significant roles in MDR, nilotinib presents a novel potential strategy for overcoming MDR in sarcoma. In another impressive prospective phase III trial performed by the Italian Sarcoma Group in collaboration with the Spanish Sarcoma Group, researchers enrolled 328 high-risk localized STS patients and randomized each to receive either three or five cycles of epirubicin plus ifosfamide⁵⁸. The group demonstrated that expression of ABCC1 (MRP1) was the only independent prognostic factor for both relapse-free survival and overall survival as compared with expression of ABCB1 and glutathione S-transferase pi.

FAK is vital to cell progression, including adhesion, growth, and survival. Overexpression and activity FAK was demonstrated in Ewing sarcoma⁵⁹. Targeting FAK with the kinase inhibitor PF-562271 and shRNA inhibited Ewing sarcoma cell growth *in vitro* and *in vivo*. High expression of FAK in osteosarcoma cells was associated with a stronger migration ability, which could be suppressed by targeting FAK with selective siRNA⁶⁰. The combination of targeting FAK by shRNA with cisplatin showed enhanced apoptosis in osteosarcoma cells⁶¹.

JAK is located intracellularly and is activated by receptor tyrosine kinases and transduce cytokine-mediated signals, which subsequently activate STAT transcription factors⁶². High levels of activated JAK/STAT3 were found in rhabdomyosarcoma, Ewing sarcoma, and osteosarcoma tumor samples and cell lines associated with poorer prognosis^{63, 64}. Targeting JAK by small molecule kinase inhibitor AZD1480 inhibited rhabdomyosarcoma and Ewing sarcoma growth *in vitro* and xenografts *in vivo*⁶³. Moreover, targeting JAK with siRNA sensitized FGF-2-mediated cisplatin resistance and inhibited cell proliferation in osteosarcoma cells⁶⁵. In addition, inhibitor of STAT3 CDDO-Me reduced resistance to doxorubicin in MDR osteosarcoma through down-regulate STAT3-mediated antiapoptotic proteins in both dose and time dependent manner⁶⁶.

Targeting serine/threonine protein kinases to reverse MDR in sarcoma

Serine/threonine kinases (STKs) are enzymes that phosphorylation the OH group of serine or threonine, which have similar side-chains. There are at least 125 kinds of STKs, which can be divided into several groups, including the TKL group, AGC group, CMGC group, CAMK group, CK1 group, RGC group, PKL group, atypical group, and Other group. There is increasing evidence that shows that STKs play a paramount role in maintaining cellular homeostasis and cell signaling transduction, and regulation of cell proliferation, differentiation, apoptosis, and embryonic development⁶⁷. DNA damage and reactive oxygen species (ROS) stress, such as cAMP/cGMP and Ca²⁺/calmodulin, can regulate activity of STKs. Dysregulation of STKs has been implicated to be associated with tumor growth, metastasis, recurrence, and chemoresistance in a variety of sarcomas⁶⁴. Representative intracellular signal transduction of serine/threonine kinases and potential target sites in sarcomas are shown in Figure. 2. Functional mechanisms reverse MDR by targeting serine/threonine kinases in sarcomas are summarized in Table 2.

Targeting polo-like kinases

Polo-like kinases (PLKs) are a group of serine/threonine kinases, including PLK1, PLK2, PLK3, PLK4 and PLK5. PLKs play important roles in the regulation of cell cycle progression through the G2/M transition and mitosis. Among PLKs, PLK1 (Polo-like kinase 1) is the most comprehensively characterized member of the PLK family. PLK1 is highly expressed in proliferative tissues, such as embryonic tissue, testis, and bone marrow. Overexpression of PLK1 increases centrosome size and/or number and aneuploidy, which contributes to tumorigenesis⁶⁸. PLK1 is overexpressed in several sarcomas, such as osteosarcoma, liposarcoma, Ewing sarcoma, and rhabdomyosarcoma, and is associated with poor prognosis^{69–72}. Targeting PLK1 with the small molecule kinase inhibitor BI 2536 significantly decreased osteosarcoma growth *in vitro* and *in vivo*⁷⁰.

PLK1 expression was significantly higher in osteosarcoma cell lines, as well as clinical specimens compared with normal muscle tissues, which was associated with a worse clinical prognosis⁷³. Targeting PLK1 with small molecule kinase inhibitor NMS-P937 reduced osteosarcoma cell growth, as well as decreased cloning efficiency and migration ability. Furthermore, combination of NMS-P937 with doxorubicin exhibited a synergistic effect in chemoresistant osteosarcoma cell lines through increased apoptosis and cell cycle arrest. More importantly, NMS-P937 showed reversal effects on doxorubicin-resistant osteosarcoma cell lines with overexpression of ABCB1 by interfering with ABCB1 mediated efflux activity, which resulted in intracellular accumulation of chemotherapeutic drugs. Recently, another study demonstrated efficacy of using RNAi to target PLK1 in the treatment of osteosarcoma. The combination of PLK1 shRNA/PEI-Lys with doxorubicin also showed marked synergistic effects on osteosarcoma cells via induced apoptosis and cell cycle arrest *in vitro* and *in vivo*⁷⁴. The combination of another PLK1 small molecule kinase inhibitor BI 6727 with vincristine showed synergistic inhibition effects in Ewing sarcoma through the induction of mitochondrial pathway apoptosis and triggered mitotic arrest⁷². In rhabdomyosarcoma, BI 2536 and BI 6727 also synergistically induced apoptosis and mitotic arrest with co-treatment with vincristine *in vitro* and *in vivo*⁷⁵. The group also confirmed

that the combination of BI 2536 with eribulin showed synergistic inhibition effects in rhabdomyosarcoma⁷¹. These findings present targeting PLK1 as a promising therapeutic strategy to reverse MDR in sarcoma.

Targeting JNK kinase

JNKs were originally identified as serine/threonine protein kinases that phosphorylate c-Jun on serines 63 and 73 in the amino-terminal activation domain. JNKs consist of JNK1, JNK2, and JNK3 belonging to the MAPK family, encoded by the MAP8, MAP9, and MAP10 genes, respectively. As the final kinases of the MAPK cascade, JNKs are responsible for integrating multiple signals and regulating cell proliferation, differentiation, transcription, and development processes. They also play a role in T-cell differentiation and the cellular apoptosis pathway⁷⁶. JNK was found to be expressed in various sarcomas, including Ewing sarcoma, osteosarcoma, chondrosarcoma, synovial sarcoma, and fibrosarcoma^{77–81}. Targeting JNK with arsenic exhibited a potential inhibitive effect on Ewing sarcoma⁷⁷. Moreover, the JNK signaling pathway is correlated with autophagy in rhabdomyosarcoma⁸². To date, two types of JNK inhibitors have been developed: peptide-based and small molecule ATP-non-competitive inhibitors, which target the upstream kinases or the downstream substrates and scaffolding proteins⁷⁶. JNK-interacting protein-1 (JIP1) is a scaffolding protein and belongs to a larger group of scaffold proteins, which also includes JIP2, JIP3, and JIP4. JIP1 selectively binds MKK4/7 (MAP kinase kinase 4, 7), MLK3 (mixed-lineage kinase 3), and JNK, which subsequently forms a functional JNK-signaling complex and regulates JNK activation, as well as JNK phosphorylation of c-Jun⁸³.

Recently, a study demonstrated that BI-78D3, an ATP-non-competitive JNK inhibitor, blocked JIP1-JNK binding and sensitized osteosarcoma cells to doxorubicin⁷⁸. The group investigated 71 osteosarcoma specimens, of which 55 samples were JIP1 positive and associated with poor prognosis. Furthermore, they found that JIP1 was more highly expressed in human osteosarcoma cell lines than in primary osteoblasts, and the combination of BI-78D3 with doxorubicin decreased JNK and JNK-JIP1 complex expression levels. In addition, BI-78D3 enhanced doxorubicin-induced apoptosis by inhibiting the anti-apoptotic JNK signaling pathway. Overexpression of c-Jun in chemoresistant tumor cells and activated c-Jun promoted the expression of ABCB1, which suggested that JNK inhibition may be related to ABCB1 in overcoming MDR⁸⁴.

Targeting cyclin-dependent kinases

Cyclin-dependent kinases (CDKs) are a family of STKs, which belong to the CMGC group and include 21 CDKs (CDK1-21) and five CDK-like (CDKL) kinases based on sequence similarity⁸⁵. CDKs play a crucial role in regulating the progression of the cell cycle. When a CDK binds a cyclin, a regulatory subunit protein, a CDK-cyclin complex is formed and the presence of CDK-activating kinase (CAK) activates kinases. CDK-cyclin complexes tightly control and monitor four sequential phases of cell cycle namely, G1, S, G2, and M phase, and dysregulation in any phase results in uncontrolled cell growth and tumorigenesis⁸⁶.

Cyclin D-dependent CDK4 and CDK6 are found to be expressed and active in rhabdomyosarcoma⁸⁷. Targeting with the small molecule kinase inhibitor PD0332991

decreased rhabdomyosarcoma *in vitro* and *in vivo* through cell cycle G1 arrest⁸⁷. CDK4 was also overexpressed in liposarcoma and associated with significantly poorer prognosis compared with the low-CDK4 group⁸⁸. A Phase II trial reported that PD0332991 could inhibit tumor cell growth and improve progression-free survival rates in patients with a CDK4 amplification and Rb expression in liposarcoma⁸⁹. In another notable report, flavopiridol, a pan-CDK inhibitor that specifically targets CDK2, CDK4, CDK6, and CDK9, inhibited liposarcoma growth⁹⁰. More importantly, flavopiridol exhibited significant enhancement of doxorubicin effects *in vitro* and *in vivo* through cell-cycle arrest and subsequent apoptosis. Furthermore, the group conducted a Phase I clinical trial of the combination of flavopiridol with doxorubicin for the treatment of advanced sarcomas, including leiomyosarcoma, fibrosarcoma, and rhabdomyosarcoma. The results also showed synergistic effects on STS. Another study demonstrated that targeting CDK1/CDK2 with the small molecule kinase inhibitor dinaciclib or siRNA induced apoptosis in osteosarcoma, fibrosarcoma, rhabdomyosarcoma, Ewing sarcoma, and liposarcoma, even in doxorubicin-resistant osteosarcoma⁹¹.sarcomas.

Targeting PI3K/AKT/mTOR

PI3K belongs to a family of lipid kinases, which phosphorylates the hydroxyl group at position 3 in the inositol ring to produce phosphatidylinositol 3-phosphate (PIP), phosphatidylinositol (3,4)-bisphosphate (PIP2), and phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Tyrosine kinase receptors (e.g. PDGFR, EGFR, IGF-IR) and G protein-coupled receptors activate PI3K as a second messenger that regulates diverse cellular functions, such as cell growth, proliferation, differentiation, membrane transport, cytoskeletal remodeling, and survival⁹². AKT (also known as Protein kinase B, PKB) is a serine/threonine protein kinase and plays a key role in regulating cell glucose metabolism, apoptosis, cell proliferation, survival, transcription, migration, and angiogenesis. AKT is a downstream mediator of the PI3K pathway, and is fully activated through simultaneous phosphorylation at two key sites: Thr308 (phosphorylated by PDK1) and Ser473 (phosphorylated by mTORC2)⁹³. There is a broad range of substrates phosphorylated by AKT, such as forkhead box O1 (FOXO1), glycogen synthase kinase 3 (GSK3), Raf, ASK, and MDM2. mTOR is also a serine/threonine protein kinase and presents a catalytic subunit of two structurally and functionally distinct signaling complexes: mTORC1 and mTORC2⁹⁴. mTOR is a central component of cellular processes that regulates cellular metabolism, growth, and survival through input from upstream pathways and in response to diverse external stimuli, including growth factors (such as IGF-1 and IGF-2), hormones, amino acids, nutrients, oxygen, energy, and stress signals. There are more than 800 proteins phosphorylated directly or indirectly by mTOR. Mutations in PTEN and dysregulation of upstream effectors (e.g. PI3K, AKT, EGFR, FGFR, KRas, MET) and downstream effectors (e.g. 4EBP1, S6K) contribute to constitutive activation of mTOR signaling, which is involved in the initiation and development of sarcomas⁹⁵. To date, mutation or constitutive activation of PI3K/AKT/mTOR has been found in most types of sarcomas, including osteosarcoma, rhabdomyosarcoma, Ewing sarcoma, synovial sarcoma, and liposarcoma⁹⁶. As a convergence point of many signaling pathways for many sarcomas, as well as a poor prognostic factor, especially relating to MDR, targeting PI3K/AKT/mTOR represents an attractive therapeutic strategy.

PI103, a dual PI3K/mTOR kinase inhibitor, can reverse MDR in fibrosarcoma and rhabdomyosarcoma⁹⁷. A study demonstrated that PI103 increased intracellular doxorubicin accumulation through decreased expression of ABCB1 and ABCC1. Furthermore, a specific inhibitor targeting the PI3K kinase, GDC-0941, showed inhibition of rhabdomyosarcoma cell growth. However, the combination of GDC-0941 with doxorubicin did not show synergistic effects. Another dual PI3K/mTOR kinase inhibitor NVP-BEZ235 did, on the other hand, show synergistic effects with chloroquine to induce apoptosis in rhabdomyosarcoma at subtoxic concentrations⁹⁸. BYL719, a novel α -specific PI3K inhibitor, combined with mafosfamide and ifosamide demonstrated a striking synergistic inhibition effect in osteosarcoma by inducing apoptosis⁹⁹. Sirolimus, which targets the mTOR pathway by directly binding to mTORC1, combined with gemcitabine enhanced antitumor growth activity in leiomyosarcoma compared with treatment by the individual single-agent drugs *in vitro* and *in vivo*^{100, 101}. Moreover, the same group conducted a Phase I study that demonstrated favorable results using the combination treatment in patients with advanced solid tumors, including chondrosarcoma. Another study demonstrated that specifically targeting mTORC2 using the kinase inhibitor PP242 or siRNA markedly promoted cisplatin-induced apoptosis and inhibited osteosarcoma cell migration ability¹⁰². Besides small molecule kinase inhibitor and siRNA, complementary targeting of the 3'-untranslated region (UTR) of mTOR by miRNA-100 also sensitized chondrosarcoma cells to cisplatin¹⁰³. In all, targeting PI3K/AKT/mTOR emerges as a promising strategy for overcoming MDR in sarcomas.

Targeting other serine/threonine protein kinases

Aurora kinases are classified between aurora kinase A, aurora kinase B, and aurora kinase C, and play a pivotal role in regulating cell mitosis and cell proliferation. Aurora kinase A and B are overexpressed in various sarcomas, including chondrosarcoma, osteosarcoma, synovial sarcoma, and Ewing sarcoma, and are associated with poor prognosis and metastasis^{104–107}. Targeting aurora kinase A and B with small molecule kinase inhibitors, shRNA, and siRNA exhibited inhibition of tumor cells^{105, 107}. Moreover, a study demonstrated that the combination of aurora kinase A and B kinase inhibitor VX-680 (Tozasertib) with etoposide and doxorubicin showed synergistic effects in Ewing sarcoma¹⁰⁷. Recently, another report also confirmed that the combination of VX-680 with cisplatin, doxorubicin, and methotrexate exhibited a synthetic lethal interaction in osteosarcoma cell lines. In addition, the synergistic effect of the combination of VX-680 with cisplatin was demonstrated in cisplatin-resistant osteosarcoma cells¹⁰⁸. The combination of aurora kinase inhibitor MSC1992371A with gemcitabine was investigated in a Phase I study in patients with solid tumors, including in sarcoma patients¹⁰⁹.

ERK belongs to the MAPK family, which is a downstream component of the MAPK cascade, known as Ras/Raf/MEK/ERK. The pathway relays signals from the cell surface for growth, stress, and other responses, and is implicated in cell survival, growth, and apoptosis in response to DNA damaging stress. Aberrant activation and/or overexpression of upstream signaling components constitutively activate ERK, which is often exhibited in different types of sarcomas, and targeting this pathway demonstrates an inhibition effect on tumors¹¹⁰. Sorafenib, a multitarget kinase inhibitor, in combination with cisplatin showed a

synergistic effect in osteosarcoma cells through downregulation of ERK¹¹¹. Targeting ERK signaling by inorganic phosphate (Pi) also exhibited sensitization to doxorubicin in osteosarcoma cells¹¹².

Checkpoint kinase 1 (CHK1) is associated with DNA damage repair, cell cycle checkpoint, and cycle arrest. The WEE1 G2 checkpoint kinase (WEE1) is also a key regulator of cell cycle progression involved in regulating the cell G2/M checkpoint entry into mitosis, cell size checkpoint, and DNA damage checkpoint. Constitutive activation and/or overexpression of CHK1 and/or WEE1 in sarcomas are implicated in tumor cell proliferation, apoptosis, and especially contribute to anticancer therapy resistance due to DNA damage repair ability¹¹³. Targeting WEE1 by kinase inhibitor MK-1775 inhibited the growth of various sarcomas, including osteosarcoma, Ewing sarcoma, and fibrosarcoma¹¹⁴. Moreover, the combination of MK-1775 with gemcitabine showed a synergistic effect *in vitro* and *in vivo* through increased apoptosis¹¹⁵. In addition, targeting CHK1 by kinase inhibitor LY2603618 increased cisplatin sensitivity in osteosarcoma¹¹⁶.

Conclusions

Sarcomas are rare malignancies associated with poor prognosis. Chemotherapy has been implemented for the last several decades and has achieved modest benefits in patients with STS. However, the development of MDR in tumor cells limits the curative potential of chemotherapeutic agents and presents challenges to the patients and the oncology team. Advances in the understanding of the molecular mechanisms of sarcomas over the past years have revealed broader roles for protein kinases in oncogenesis and the development of sarcomas, particularly in regard to MDR. Tremendous research and clinical trials have shown that targeting protein kinases by small molecule agents, antibodies, RNAi, and in combination with conventional chemotherapy represents a promising therapeutic approach to overcoming MDR in sarcoma.

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Highlights

1. MDR attenuates the efficacy of anticancer drugs and results in treatment failure for sarcomas.
2. Certain protein kinases demonstrate aberrant expression and/or activity in sarcoma cells, which have been found to be involved in the MDR of sarcoma.
3. Targeting protein kinases in combination with conventional chemotherapy represents a promising therapeutic approach to overcoming MDR in sarcoma.

Figure 1. Targeting tyrosine kinases reverse MDR in sarcomas

Intracellular signal transduction of tyrosine kinases and potential target sites in sarcomas, including receptor tyrosine kinases, non-receptor tyrosine kinases, ABC transporters, and three major downstream signaling pathways: PI3K/AKT/mTOR, MAPK, and JAK/STAT pathways. Bold represent target tyrosine kinases by small molecule kinase inhibitors and mAb.

Figure 2. Targeting serine/threonine kinases reverse MDR in sarcomas

Intracellular signal transduction of serine/threonine kinases and potential target sites in sarcomas, including serine/threonine kinases, ABC transporters, cell cycle, and two major downstream signaling pathways: PI3K/AKT/mTOR and MAPK pathways. Bold represent target serine/threonine kinases by small molecule kinase inhibitors.

Table 1

Targeting tyrosine kinase reverse MDR in sarcomas

Target Kinase	Inhibition compound	functional mechanism	Effect on anticancer drug	Sarcoma	References
EGFR	Gefitinib	apoptosis, cell-cycle arrest, ABCC1	Vincristine	Leiomyosarcomas	9
	Erlotinib	apoptosis, cell-cycle arrest	Cisplatin	Chondrosarcoma	16
	Cetuximab	natural killer cells	Doxorubicin, methotrexate	Osteosarcoma	17
AXL	BGB324	PI3K/AKT, ERK pathways	Vincristine, doxorubicin	Ewing sarcoma	24
VEGF	Bevacizumab	VEGF	Gemcitabine, docetaxel	Leiomyosarcoma, angiosarcoma, liposarcoma	41
PDGFR	Imatinib	Reactive oxygen species accumulation	Doxorubicin	Osteosarcoma	38
	Sorafenib	VEGFR, PDGFR, Raf	Topotecan	Osteosarcoma, Ewing sarcoma	4
MET	Anti-HGF antibody	PI3K/AKT pathway	Cisplatin	Osteosarcoma	45
	PHA-665752	PI3K/AKT pathway	Cisplatin	Osteosarcoma	45
	Chinaphilin	apoptosis	Doxorubicin	Osteosarcoma	34
IGF-1R	AG1024	apoptosis, cell cycle arrest, cytotoxicity	Doxorubicin	Osteosarcoma	32
	AVE1642	apoptosis	Gemcitabine	Leiomyosarcoma	31
SRC	Dasatinib	apoptosis	Doxorubicin, vincristine, doxorubicin, actinomycin D	Chondrosarcoma synovial sarcoma	57,50
BCR-ABL _c -KIT, PDGFR	A-770041	apoptosis, ABCB1	Paclitaxel, doxorubicin	Osteosarcoma	56
	Nilotinib	ABCB1, ABCC1	Doxorubicin	Synovial sarcoma, leiomyosarcoma	7
JAK	CDDO-Me	apoptosis, JAK/STAT pathway	Doxorubicin	Osteosarcoma	66

Table2

Targeting Serine/threonine kinase reverse MDR in sarcomas

Target Kinase	Inhibition compound	functional mechanism	Effect on anticancer drug
Polo-like kinase 1	BI 6727	apoptosis, cell cycle arrest	Vincristine
	BI 2563	apoptosis, cell cycle arrest	Vincristine, Eribulin
	NMS-P937	apoptosis, cell cycle arrest, ABCB1	Doxorubicin
JNK	BI-78D3	apoptosis	Doxorubicin
	Flavopiridol	apoptosis, cell cycle arrest	Doxorubicin
CDK	Dinaclitib	apoptosis	Doxorubicin
PI3K	BYL719	apoptosis	Mafofamide, ifosamide
Dual PI3K/mTOR	PI103	ABCB1, ABCC1	Doxorubicin
	PP242	apoptosis	Cisplatin
	Sirolimus	apoptosis	Gemcitabine
Aurora kinase	Tozasertib	apoptosis, cell cycle arrest	Etoposide, doxorubicin
	Sorafenib	ERK	Cisplatin
	MK-1775	apoptosis	Gemcitabine
CHK1	LY2603618	apoptosis, cell cycle arrest	Cisplatin