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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 chemotherapeutics 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.

Introduction

Sarcomas are a heterogenous 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 3000 cases of bone sarcomas [1]. The 5-year overall survival rate is approximately 50–80% for sarcomas [2].

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, 30–40% of patients will experience MDR associated with recurrence or metastasis despite improved multimodality therapy [3]. 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

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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 MDR [4].

The human kinome contains at least 600 protein kinases that carry out the phosphorylaton 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 use [5]. 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 tumors [6]. 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 targets [7].

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 apoptosis [8]. 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 MDR [9]. 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 rhabdomyosarcoma [10]. Targeting tyrosine kinases can not only lead to inhibition of these kinases and down-regulation of downstream signal pathways to decrease tumor cell growth, but may also present a potential strategy to reverse MDR in sarcoma (Fig. 1 and 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 PLCγ/PKC pathways, leading to DNA synthesis and regulation cell proliferation, adhesion, and differentiation [11]. 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 prognosis [12]. 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 MDR [9]. 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 chemotherapy [15]. 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