The importance of Src signaling in sarcoma (Review)

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Abstract. Src is a tyrosine kinase that is of significance in tumor biology. The present review focuses on Src, its molecular structure, and role in cancer, in addition to its expression and function in sarcoma. In addition, the feasibility of Src as a potential drug target for the treatment of sarcoma is also discussed. Previous studies have suggested that Src has essential functions in cell proliferation, apoptosis, invasion, metastasis and the tumor microenvironment. Thus, it may be a potential target for cancer therapy. Src has been found to enhance proliferation, reduce apoptosis and promote metastasis in certain subtypes of sarcoma, including osteosarcoma, chondrosarcoma and Ewing's sarcoma. Furthermore, a number of novel effective therapeutic agents, such as SI-83, which target Src have been investigated *in vitro* and *in vivo*. Bosutinib and dasatinib, which inhibit Src, have been approved by the U.S. Food and Drug Administration for the treatment of chronic myelogenous leukemia. In addition, vandetanib is approved for the treatment of medullary thyroid cancer. Furthermore, the Src inhibitor, saracatinib, is currently in clinical trials for the treatment of a variety of solid tumors, including breast and lung cancers. Thus, Src is considered to be an important factor in sarcoma progression and may present a novel clinical therapeutic target. This review demonstrates the importance and clinical relevance of Src in sarcoma, and discusses a number of small molecular inhibitors of src kinase, such as dasatinib and sarcatinib, which are currently in clinical trials for the treatment of sarcoma patients.

Contents

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1. Introduction

Sarcoma is a soft-tissue and bone malignancy of mesenchymal origin, which accounts for ~1% of adult cancers and 15-20% of pediatric cancers in the USA (1,2). In the USA, ~11,280 soft tissue tumors and 2,650 bone tumors are diagnosed annually (3). Due to the heterogeneity of sarcoma, >100 distinct subtypes have been described to date, with new subtypes frequently reported (4). Synovial sarcoma, a soft-tissue tumor, is characterized by a reciprocal t(X;18) translocation, in which the SS18 gene on chromosome 18 fuses with the SSX1, SSX2 or, less commonly, SSX4 gene on the X chromosome (5,6). Ewing's sarcoma has a relatively simple genetic signature, consisting of a t(11;22) translocation (7,8). However, certain other sarcomas, including osteosarcoma, chondrosarcoma and undifferentiated sarcoma, are characterized by more complex genetic abnormalities (9).

The clinical outcomes of sarcoma are dependent upon the subtype, and current therapies are limited to radiation, chemotherapy and surgical resection. Although radiation may prevent local recurrence, and chemotherapy can temporarily delay the progression of sarcoma, complete surgical resection is the only curative treatment method (10,11). As the rate of complication and of chemotherapy resistance are considerable, a more effective therapy is urgently required (12). During the last two decades, many of the molecular mechanisms of sarcoma genesis have been elucidated; novel insights into such mechanisms, and the identification of the involved genes may lead to the development of more effective therapies targeted against the driving events in sarcomas (13).

In the current review, the structure of Src and its function as an oncoprotein are described, with a detailed discussion of the role of Src in sarcoma. In addition, potential drug therapies for the treatment of sarcoma are also evaluated.

2. Src

Src structure and regulation of Src activity. SRC is a proto-oncogene encoding a non-receptor tyrosine kinase, similar to the v-Src gene of the Rous sarcoma virus (14), which was initially discovered by Bishop and Varmus (15). The Src protein is

formed of seven functional regions: i) N-terminal Src homology domain 4 (SH4) containing a myristic acid moiety, essential for its localization to the inner surface of the cell membrane; ii) a unique domain providing functional specificity to each member of the Src family; iii) SH3 domain, which binds proline-rich sequences to mediate intra- and intermolecular interactions; iv) SH2 domain, which binds phosphorylated tyrosine residues on Src and other proteins; v) a catalytic domain (SH1); and vi) C-terminal tail containing negative-regulatory Tyr530 (in humans) (16-18) (Fig. 1).

The activity of Src is regulated by the structural changes that occur following phosphorylation and dephosphorylation of its tyrosine residues, which is determined by the relative activities of protein kinases and phosphatases (19). The enzymatic activity of the 60 kDa human c-Src tyrosine kinase is predominantly regulated at two phosphorylation sites: Tyr527 and Tyr416. Phosphorylation at Tyr527 reduces the activity of Src, while dephosphorylation of phosphotyrosine 527 increases activity; autophosphorylation of Tyr416 also enhances activity (20,21). Phosphatases that may interact with phosphotyrosine 527 include cytoplasmic protein tyrosine phosphatase (PTP) 1B, Shp1 and Shp2, and transmembrane enzymes including CD45, PTPα, PTPε, and PTPκ (22,23). Furthermore, PTP-BL and PTP-BAS have been shown to dephosphorylate phosphotyrosine 416 to decrease Src kinase activity (24) (Fig. 2).

Functions of Src in cancer. Src has been identified as an important factor in several human malignancies, and in the promotion of tumor progression during the multistep process of cancer pathogenesis (25). Src deregulation primarily involves protein overexpression and abnormalities in Src kinase activity. Differences in Src expression have been observed in lung, breast, pancreatic, colon and prostate cancer cells, compared with normal adjacent tissue, fibroblasts or normal mucosal cells (26-31). In the tumor microenvironment, Src activation has been observed in cancer and inflammatory cells, and may serve as a critical mechanistic link between inflammation and cancer. Src propagates a cycle between immune and tissue cells, ultimately leading to the development and progression of cancer (32,33). The abnormal activation of Src may result in the promotion of survival, angiogenesis, proliferation and invasion pathways observed in tumors cells (34,35). However, despite the evidence indicating a major role for Src in the development and progression of cancers, its mechanism of action is not fully understood.

A number experimental studies have proposed that Src may be involved in the transmission of signals from extra and intracellular stimuli. Interactions between the Src pathway and Signal Transducer and Activator of Transcription (STAT) 5, STAT3, N-cadherin and basic fibroblast growth factor receptors and β-catenin have been reported in melanoma cells (36,37). It may also be of value to understand the effect of Src inhibition on a number of the environment-sensing and growth-promoting pathways known to be aberrant in cancer cells, including the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR), Ras/mitogen-activated protein kinase (MAPK), platelet-derived growth factor (PDGF), Erb1/Erb2 and vascular endothelial growth factor (VEGF) pathways (38-40). Currently, the complex interactions between Src and other pathways remain to be established. The crosstalk signaling mechanisms that link inflammatory cells with cancer cells, including SDF-1-CXCR4-Src and Src-IL-6 signaling axes, result in a cycle leading to cancer development and progression (41-43). In leukemia, SDF-1 has been found to induce 'inside-out' signaling, which involves CXCR4 and Lyn, leading to aberrant adhesive responses. Furthermore, previous studies have shown that Src and Hck, the Src family members, are involved in the production of IL-6 in osteoblasts and inflammatory macrophages (42,43).

3. Function of Src in sarcoma

Src aberrant expression in sarcoma. Src was the first transforming protein and the first gene product with protein tyrosine kinase activity to be discovered and isolated (44). With the use of immunohistochemistry and Western blotting, the total Src and phosphorylated Src (Y419) were found to be activated in human sarcoma tissues (leiomyosarcoma, high-grade osteosarcoma and liposarcoma) and sarcoma cell lines (osteosarcoma, Ewing's sarcoma, leiomyosarcoma and rhabdomyosarcoma) (45). Furthermore, Src was identified as one of the most strongly phosphorylated kinases in synovial sarcoma cells (46). Src activity was demonstrated to be upregulated in anoikis-resistant human osteosarcoma cells, SAOS-2, compared with their parental population (47).

With regard to different subtypes of sarcoma, Src is thought to be the most reliable discriminator to distinguish high-grade leiomyosarcoma from undifferentiated pleomorphic sarcoma, based on gene expression profiling and meta-analysis (48). Due to its aberrant expression in sarcoma, Src has been proposed to be important in signal transduction in human sarcomas, including osteosarcoma, rhabdomyosarcoma, leiomyosarcoma, fibrosarcoma and Ewing's sarcoma (49).

Src in sarcoma proliferation and apoptosis. A fundamental trait of cancer cells is their ability to sustain chronic proliferation. The overexpression of Src in U2OS and MG63 osteosarcoma cells significantly enhances proliferation and reduces apoptosis of these cells (45,50). In human osteosarcoma cells SAOS-2, Src was revealed to be activated in anoikis resistance (47). Furthermore, Src was identified in 0-20% chondrosarcoma specimens. However, its expression had no prognostic significance, particularly in serving as an indicator of cell proliferation (51). Src and its downstream signaling via the p38 MAPK-AKT pathway may be activated by the signaling adaptor protein, Crk, to promote proliferation of human synovial sarcoma cells (52,53). Inhibition of Src signaling in Ewing's sarcoma cells was observed to induce apoptosis (45).

These findings indicate that Src may increase sarcoma proliferation and reduce apoptosis. However, in some subtypes of sarcoma, there is conflicting evidence with regard to the expression of Src. For example, high Src expression has been identified in high-grade leiomyosarcoma, while Src expression has been found to be variable in chondrosarcoma (48). Additionally, the mechanisms of proliferation and apoptosis require further investigation.

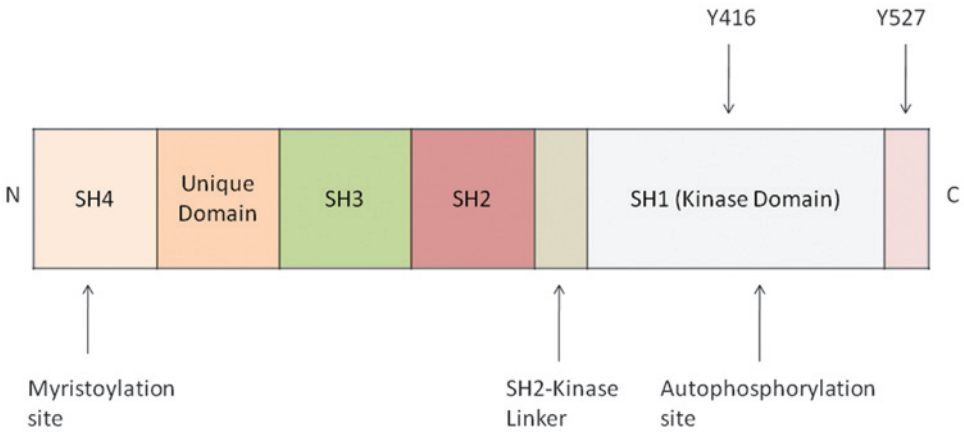

Figure 1. Structure of human c-Src, comprising seven functional domains. SH, Src homology.

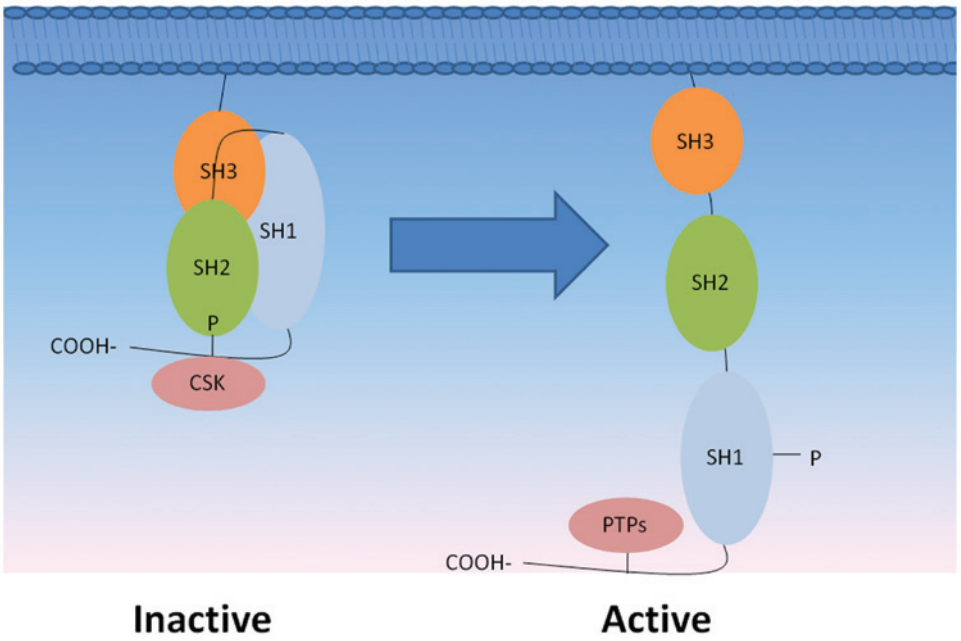

Figure 2. Schematic representation of Src in the inactive (left) and active (right) states. The N-terminus binds to the cell membrane. The SH3 domain forms interactions with the linker between the SH2 and the SH1 domain. The SH2 domain binds the phosphorylated C-terminal tail, and these two alterations prevent the SH1 domain from being phosphorylated at Y419 and reduce the potential for substrate interactions. SH, Src homology; CSK, C-Src kinase; PTP, protein tyrosine phosphatase.

Src in sarcoma invasion and metastasis. Despite continual research and increasing knowledge of the biology of sarcoma, invasion and metastasis remain poorly understood, and are the predominant cause of sarcoma-related mortality. The ability of cancer cells to leave their primary site of growth, move into different tissue compartments, and survive and proliferate in these foreign environments, defines the biological program known as 'invasive growth' (54). Invasive growth is important for cancer progression and thus, presents a target for the treatment of sarcoma. In mouse models of osteosarcoma, depletion of Src phosphorylation in SaOS-2 cells has been shown to decrease tumor mass (55). However, other reports indicate that inhibition of Src phosphorylation in HOS and SaOS-2 cells may only decrease the metastatic potential of osteosarcoma cells *in vitro*, and not *in vivo* (56). The effect of Src on the metastasis of osteosarcoma cells is therefore controversial. A number of studies reported that inhibition of c-Src signaling was able to reduce metastasis of chondrosarcoma (57,58). Other studies found that Src inhibition could overcome chemoresistance to induce apoptosis and to inhibit migration (59). In Ewing's sarcoma cells, inhibition of c-Src was also observed to reduce migration and metastasis (45).

It has been established that epithelial cells may acquire migratory capability, a feature typical of the mesenchymal cells, and gain invasive ability, resistance to apoptosis and the ability to disseminate (60), in a process known as the epithelial-mesenchymal transition (EMT). EMT is a complicated process, whereby cancer cells acquire migratory and invasive abilities, which are influenced by the tumor microenvironment and intercellular communication. Src activity affects metastatic progression, suggesting that Src-induced EMT may be associated with enhanced metastatic potential (61). However, the effect of Src-related EMT has yet to be investigated in sarcoma.

4. Src signaling networks in sarcoma

A number of studies have provided insight into how Src overexpression and activation may contribute to cancer. CD99, a transmembrane glycoprotein, may exert anti-oncogenic effects, reducing the growth and metastatic ability of osteosarcoma cells by regulating Caveolin-1 (Cav-1) and inhibiting Src kinase activity. Cav-1 is a caveolar domain associated with the plasma membrane, which is involved in numerous cellular functions, including molecular transport, cell adhesion and signal transduction and thus, the role of Cav-1 in cancer development and progression has been investigated (62,63). Cav-1 may act as an onco-suppressor and inhibit Src to reduce osteosarcoma metastasis (64,65). However, other studies have demonstrated that CD99 isoforms, CD99wt (full-length CD99 isoform) and CD99sh (short form) have opposing effects in osteosarcoma malignancy and metastasis, and may activate or inhibit Src kinase activity (66).

In osteosarcoma, when Src was inhibited, the downstream components of Src signaling, including focal adhesion kinase (FAK) and a partnership and Crk-associated substrate (p130CAS) were also inhibited at the protein level. In rhabdomyosarcoma, targeting the Src-α-type platelet-derived growth factor receptor-Raf-MAPK axis has been shown to be effective in inhibiting mouse and human tumor cell growth (67).

5. Clinical development of Src inhibitors

Src has recently become an active target for drug development and a number of Src inhibitors, including dasatinib (BMS354825), sarcatinib (AZD0530) and bosutinib (SKI-606), are at various stages in the development process (68). Dasatinib has been approved for the treatment of chronic myeloid leukemia and Philadelphia-positive acute lymphoblastic leukemia (69), saracatinib has been used in a phase II trial for the treatment of extensive stage small cell lung cancer (70), and bosutinib has been used in a phase II trial for the treatment of adults with recurrent glioblastoma (71).

Dasatinib is a dual Src-AbI kinase inhibitor, which is already approved by the Food and Drug Administration for the treatment of chronic myeloid leukemia and Philadelphia chromosome positive acute lymphoblastic leukemia (72). Several studies have demonstrated the therapeutic benefit of dasatinib in preventing the growth and metastasis of sarcomas. In osteosarcoma cell lines, wound-healing, cell migration and TUNEL assays indicated that dasatinib may block cell motility and invasion, and induce apoptosis (45,73). In chondrosarcoma, dasatinib was also capable of decreasing tumor growth, however, it was unable to reduce invasion (73).

A new pyrazolo[3,4-d] pyrimidine derivative Src-Y416 inhibitor (SI-83) was found to impair osteosarcoma SaOS-2 cell viability and decrease osteosarcoma tumor mass *in vivo*, and exhibited less toxicity in primary human osteoblasts when compared with osteosarcoma cells. Additionally, SI-83 was shown to induce apoptosis in SaOS-2 cells (55). These results indicate that SI-83 may be a novel effective therapeutic agent, with the advantage of low toxicity in nonneoplastic cells. A number of tyrosine kinase inhibitors that target Src tyrosine kinase have also been developed for therapeutic use (74), such as the pan-RAF inhibitors, CCT196969 and CCT241161 (75).

6. Conclusion

Compared with normal tissue, Src expression is significantly higher in tumor tissue, including gastrointestinal stromal tumors and renal clear cell carcinomas (76,77). A number of studies have found that Src signaling is important in attracting immune cells to tumor cells (32). The activation of Src, mediated by inflammatory cytokines and chemokines within the tumor microenvironment, occurs in cancer cells and immune inflammatory cells (78,79).

However, due to the intra- and inter-tumor heterogeneity, targeting a single genetic event in sarcoma is unlikely to produce favorable clinical outcomes. Furthermore, understanding the role of Src in the initiation and progression of sarcoma is at an early stage, and the mechanisms by which Src affects the sarcoma microenvironment and the immune system remain to be investigated. Optimal treatment may include surgical resection combined with therapies that target the functional processes involved in tumor biology and metastasis, including chemotherapy and immunomodulation (80,81). The Src protein exhibits high specificity and a positive predictive value, highlighting its potential as a diagnostic marker for certain types of sarcoma, such as osetosarcoma and Ewing's sarcoma. Thus, Src inhibitors may present a novel type of chemotherapeutic drug for the treatment of sarcoma, however, preclinical studies to determine the optimal protein sequence for Src-targeted treatments and methods to monitor the theapeutic effects of such are required.

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