The role of Src in prostate cancer

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The Src family kinases (SFKs) are the largest family of nonreceptor protein tyrosine kinases and are responsible for signal transduction during many cellular activities, including differentiation, adhesion, and migration. Aberrant Src/SFK activity has been widely implicated in cancer development. Several lines of evidence indicate a role for SFKs in the development of prostate cancer, e.g. SFK overexpression in prostate cancer cell lines and tissues and reduced cancer cell proliferation, invasion, and migration following Src inhibition. In particular, Src may be involved in androgen-independent growth during advanced stages of disease. Src signaling is also a key pathway during normal and dysregulated bone functioning, and bone metastases are responsible for substantial morbidity in advanced prostate cancer. Src/SFK inhibition therefore represents a potentially useful therapeutic strategy for patients with various stages of prostate cancer. To date, four Src inhibitors have reached clinical trials. Of these, the broadest range of in vitro prostate cancer data are available for dasatinib, which inhibits several SFKs as well as other tyrosine kinases. Src inhibitors may be specifically evaluated in prostate cancer clinical trials in the near future.

Key words: dasatinib, prostate cancer, Src, Src family kinase, Src inhibitors

introduction

Src is the prototypical member of the Src family kinases (SFKs), the largest family of nonreceptor protein tyrosine kinases [1–4]. The SFK family comprises nine members (Blk, Fgr, Fyn, Hck, Lck, Lyn, Src, Yes, and Yrk). These proteins are responsible for signal transduction from various cell-surface receptors, including growth factor receptors, integrins and other adhesion receptors, seven transmembrane guanosine phosphate-binding protein-coupled receptors (GPCRs), cytokine receptors, immunological recognition receptors, and ion channels. SFKs are essential for many cellular activities, including cytoskeletal alterations, differentiation, cell-cycle progression, adhesion, and migration.

Src is the most widely studied of the SFKs, although all family members have similar structural features [1–4]. Src is a 60-kDa protein consisting of distinct functional regions that each contains a different Src homology (SH) domain. The aminoterminal region comprises an SH4 domain, which contains signals for lipid modification, and a unique region of low conservation thought to mediate protein interactions that are specific for each SFK. Linked to this are SH3 and SH2 domains, which are also protein binding and play a critical role in regulating Src activity. A catalytic domain (SH1) is responsible for tyrosine kinase activity and is joined to a short carboxy-terminal tail containing a negative regulatory tyrosine residue (Y530 in the human protein). When Src is inactive, the SH2 domain binds to phosphorylated Y530 and the SH3 domain binds to the catalytic domain, causing a closed conformation that prevents kinase activity (Figure 1). Y330 phosphorylation is maintained by regulatory proteins C-terminal Src kinase (Csk) and Csk-homologous kinase [5]. Src can be activated by the binding of ligand-bound cell-surface receptors or by cytoplasmic proteins, such as focal adhesion kinase (FAK) or its molecular partner Crk-associated substrate (CAS) [1, 6–8]. Binding disrupts intramolecular interactions within the Src protein, leading to an open conformation, which enables SH2 and SH3 domains to bind to downstream proteins and the catalytic domain to interact with potential substrates (Figure 1). Y330 dephosphorylation by cellular phosphatases can also induce Src activation. Full activation requires the autophosphorylation of a tyrosine residue located within the catalytic domain (Y419 in the human protein) [2].

A wide body of evidence implicates aberrant Src/SFK activity in cancer development [1–4]. A highly activated viral version of Src (v-Src), the first oncogene to be discovered, is responsible for the transforming properties of the oncogenic Rous sarcoma virus. v-Src is a constitutively active version of cellular Src lacking the C-terminal regulatory region (containing the Y530 residue) found in the cellular protein. Because of their signaling roles during normal cellular processes, SFK-activated pathways are also involved in tumor adhesion, motility, invasion, and angiogenesis (Figure 2). In addition, increased activity or expression of Src or other SFKs has been reported in several malignancies [9–14]. In colorectal cancer, Src activity is higher in metastatic tissue than in

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primary tumor tissue [15, 16] and Src activity can be predictive of poor clinical prognosis [17], indicating a potential role in tumor progression. Src and SFKs are therefore attractive targets for potential novel anticancer therapies.

Several mechanisms have been proposed to explain the increased Src activity observed in tumors. Src is downstream from a number of growth factor receptors, including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and vascular endothelial growth factor receptor [1, 6]. Several tumor types, including prostate cancer, have excessive expression or activity of these receptors or their ligands [18–21]. Src is also activated by cytoplasmic proteins, e.g. FAK, which is overexpressed in different cancers [22, 23]. Src may be aberrantly activated because of naturally occurring mutations [24, 25], although other studies have failed to detect these mutations, indicating that this cause of Src activation may be rare [26–29]. Other mechanisms of Src activation in cancer might include increased expression of activatory phosphatases [30, 31] or decreased levels of regulatory proteins, e.g. Csk [32, 33].

This review will now focus on the role of Src and SFKs in prostate cancer and on the potential use of Src inhibitors.

**Src and SFKs in prostate cancer**

Several lines of evidence indicate a role for SFKs in prostate cancer. SFK members Src and Lyn are highly expressed in prostate cancer cell lines, as well as in the majority of prostate cancer specimens (Figure 3) [34–36]. In separate studies, Src inhibitors decreased the proliferation, invasion, and migration of prostate cancer cell lines in vitro [34, 36–40]. Src inhibitors also reduced prostate cancer growth and metastasis in mouse xenograft studies [41–43]. Src signaling is involved in androgen-induced proliferation of prostate cancer

![Figure 1](image1.png)  
**Figure 1.** Activation of Src. The left panel represents the closed or inactive conformation of Src, in which Y530 interacts with the SH2 domain, positioning the SH3 domain to interact with the linker between the SH2 and catalytic domains. This results in diminished access of substrates to the kinase domain. The middle panel illustrates different mechanisms involved in the activation of Src (indicated by arrows). The right panel represents the open or active conformation. Taken from Martin [4].

![Figure 2](image2.png)  
**Figure 2.** Src-mediated pathways that may contribute to tumor progression. CAS, Crk-associated substrate; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; IKK, IκB kinase; IL-8, interleukin 8; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase MAPK/ERK kinase; MLCK, myosin light chain kinase; NFκB, nuclear factor κB; PI3K, phosphatidylinositol 3-kinase; RhoGAP Rho GTPase-activating protein; RTK, receptor tyrosine kinase; SOS, son of sevenless; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor. Taken from Summy and Gallick [141].
cells [44] and may also participate in the transition to androgen-independent growth [45–47]. Src inhibition represses androgen-independent growth and metastasis [42, 45]. Gene amplification of Fgr (an SFK) is often found in cancer tissue from patients with castration-refractory prostate cancer [48]. Aberrant SFK signaling is therefore implicated across multiple stages and models of prostate cancer.

Because SFKs are involved in numerous signaling pathways, blocking these proteins might also inhibit other proteins implicated in prostate cancer pathogenesis. For example, several growth factors and their receptors are overexpressed in prostate cancer [49, 50]. EGFR expression correlates with prostate cancer relapse and progression [51] and Src/EGFR synergism may contribute to a more aggressive tumor phenotype [52, 53]. Src has a well-recognized role in PDGFR mitogenic signaling [6, 54] and PDGFR inhibition blocked prostate cancer growth in preclinical studies [55, 56], although this remains to be demonstrated in the clinic [57–61]. Src is also likely to be involved in oncogenic signaling from vascular endothelial growth factor, insulin-like growth factor (IGF-I) and GPCRs [62–64]. The EphA2 receptor is overexpressed in prostate cancer cell lines and tissues [65, 66], and evidence indicates that SFK signaling is involved in EphA2 upregulation in noncancer tissues [67, 68]. Intracellular Src-activating proteins implicated in prostate cancer progression include FAK [38, 69] and tr-Kit [70]. These data indicate that Src is potentially involved in numerous signaling pathways that may contribute to prostate cancer.

role of Src in bone metastases from prostate cancer

Bone metastases occur in the majority of patients with prostate cancer during advanced stages of the disease [71]. When bone metastases are established, cancer cells dysregulate the normal bone maintenance and remodeling process through the secretion of growth factors and cytokines that disrupt the balanced activities of osteoclast and osteoblast cells (responsible for bone resorption and bone formation, respectively) [72–75]. Osteoclast-mediated bone resorption leads to the release of immobilized growth factors from bone, which further stimulate tumor cell proliferation, creating a 'vicious cycle' that increases both bone destruction and tumor burden (Figure 4). This results in the common morbidity of bone metastases, which includes bone pain, pathological fractures, and spinal cord compression [71]. Although prostate cancer gives rise to predominantly osteoblastic lesions in bone, as assessed by imaging techniques, it is now understood that osteoclast and osteoblast activities are linked, with almost all bone lesions being formed by a combination of increased bone resorption and formation [74, 75]. Recently, major progress has been made in understanding the cross talk between osteoblasts and osteoclasts with the identification of the receptor activator of nuclear factor kB ligand (RANKL)/osteoprotegerin axis [76]. There is now extensive evidence that metastatic cells within bone interfere with this axis [73, 77–79]. At present, specific treatments of bone metastases include bisphosphonates (mainly intravenous), localized radiotherapy, and radiopharmaceuticals [71, 80, 81]. It is also noteworthy that prostate cancer patients are at risk of developing decreased bone mineral density and related fractures following androgen deprivation therapy (ADT) [82].
matrix, including TGF-β, PTHrP. Osteolysis results in release of growth factors from the bone and transforming growth factor (TGF)β-factors, including endothelin-1 (ET-1), fibroblast growth factors (FGFs), which normally inactivates RANKL. Prostate cancer cells secrete additional factors leading to osteolysis. PTHrP also downregulates osteoprotegrin (OPG), and tumor cells activates osteoblasts to produce receptor activator of nuclear factor kB ligand (RANKL). This activates osteoclast precursors, and PTHrP by tumor cells activates osteoclasts result in both increased osteoclast activation and tumor growth. Production of parathyroid hormone-related protein (PTHrP) by tumor cells activates osteoblasts to produce receptor activator of nuclear factor kβ ligand (RANKL). This activates osteoclast precursors, leading to osteolysis. PTHrP also downregulates osteoprotegrin (OPG), which normally inactivates RANKL. Prostate cancer cells secrete additional factors, including endothelin-1 (ET-1), fibroblast growth factors (FGFs), and transforming growth factor (TGF)-β2, which have similar effects to PTHrP. Osteolysis results in release of growth factors from the bone matrix, including TGF-β and insulin-like growth factor (IGF), and raises extracellular calcium (Ca²⁺) levels, which in turn promotes tumor cell proliferation and PTHrP production. Adapted from Fizazi et al. [73] and Mundy [74].

Src signaling is a key pathway during healthy bone turnover. Although Src is ubiquitously expressed, high levels are found in osteoclasts [1]. Targeted disruption of the Src gene in mice affects only bone development; Src-deficient mice develop osteopetrosis [83], caused predominantly by a defect in osteoclast-mediated bone resorption [84]. Src kinase activity is essential for osteoclast cytoskeletal organization and bone-resorbing activity [85], and Src-deficient osteoclasts have reduced migration [86] and other functional defects [83, 87]. Src and SFKs are involved in the antiapoptotic signaling of RANKL and other tumor necrosis factor family members in osteoclasts [88–90]. Src inhibitors reduce osteoclast numbers, induce apoptosis, and impair osteoclast bone resorption, adhesion, and cytoskeleton reorganization [91–97].

An essential role for Src in osteoblasts has also been demonstrated. In mice, Src inhibition or disruption reduced osteoblast proliferation but enhanced osteoblast differentiation and bone-forming activity [98]. Src suppression also reduced the paracrine stimulation from breast cancer cells to osteoblasts for production of osteoclastogenic cytokines [99].

Src signaling may be involved in tumor metastasis to bone. In mouse breast cancer models, Src inhibition decreased the size and delayed the appearance of bone lesions [99, 100]. IGF and IGF-binding proteins have been implicated in bone metastasis development in prostate cancer [73], and Src activation is involved in the IGF-IR upregulation by androgens [63].

In summary, the central role of Src signaling in osteoclast and osteoblast activities indicates that Src inhibitors have an additional therapeutic potential in prostate cancer for decreasing the morbidity associated with bone metastases, as well as ADT-induced bone loss.

**Src and SFKs as therapeutic targets in prostate cancer**

Because of the multiple roles of Src and SFKs, targeted therapies are promising agents for several stages of prostate cancer. Careful consideration is needed for any novel oncology treatment with respect to optimal sequencing, potential combination with other treatments, and the identification of disease subgroups that may derive the greatest benefit. In prostate cancer, optimal treatment strategy is an area of debate in several disease stages [101–103].

The sensitivity of prostate-specific antigen (PSA) testing means that an increasing proportion of prostate cancer cases are detected early, when the tumor is still localized. There is now evidence from a randomized clinical trial that local treatment improves survival in early prostate cancer in the long term [104]. Because of treatment-related adverse events, patients at low risk of progression, who are likely to die of unrelated causes before the disease progresses, may be managed by active surveillance [102, 105].

Patients with high-risk localized prostate cancer are most likely to develop distant metastases and eventually die of their cancer. In this setting, ADT combined with radiotherapy has demonstrated a survival advantage in randomized trials [106–108]. There is, however, definite need for more efficacious options. Src inhibitors may represent a potentially useful adjunctive therapy following local treatment to target remaining tumor cells.

Patients with advanced prostate cancer receive ADT, reflecting the almost ubiquitous expression of the androgen receptor [80, 109]. Because of their different modes of action, Src inhibitors may be useful in combination with ADT. In addition, ADT is frequently used in patients with disease progression detectable only by a rising PSA level, although the benefits of this treatment strategy are not clear [82, 101]. There is a need to assess novel therapies such as Src inhibitors in this patient group, especially patients at high risk of developing metastases.

While on ADT, prostate cancer eventually progresses to an androgen-independent (castration-refractory) state. At present, docetaxel-based chemotherapy is the standard of care for patients with symptomatic metastatic castration-refractory prostate cancer [80, 109, 110]. There is, however, no widely accepted treatment of patients with castration-refractory disease without evidence of metastases. In addition, some elderly patients may be unable to tolerate aggressive chemotherapy. Because the median survival for patients with metastatic castration-refractory prostate cancer with recommended available treatments is ~18 months [111, 112], there is an urgent need for more effective therapies, including agents with activity against bone metastases. There is a clear potential for Src inhibitors to be used in all stages of castration-refractory disease, and clinical trials should address whether Src inhibitors may be used as single agents or in combination with current chemotherapy regimens.
Because SFK inhibitors represent novel therapies, several studies have investigated potential combination activity with other anticancer agents. Synergy with Src inhibitors has been demonstrated in various nonprostate cancer models for docetaxel, gemcitabine, platinum agents, and imatinib [113–116]. Src inhibitors are highly likely to have combined activity with several chemotherapies currently used to treat prostate cancer.

Under normal circumstances, Src appears to be a positive regulator of osteoclast activity and a negative regulator of osteoblast differentiation and bone formation. Inhibiting Src may be beneficial for patients with osteolytic lesions. Also, because radiologically osteoblastic lesions result from an overall increase in bone turnover that includes increased osteolytic activity, patients with osteoblastic lesions may also benefit. In support of this, zoledronic acid, a bisphosphonate inhibitor of osteoclast-mediated bone resorption, prevents skeletal complications in patients with metastatic prostate cancer [117]. Moreover, denosumab, a RANKL-binding mAb that inhibits osteoclast activity, has recently shown promising activity in patients with bone metastasis, including those from prostate cancer [118].

Src-targeting therapies are a recent development. Although numerous agents have been discovered [119–121], few have reached clinical development. Preliminary animal studies in tumor types other than prostate cancer are promising and indicate that Src inhibitors decrease tumor growth and metastasis [99, 122, 123], Src inhibitors currently in clinical trials, or with activity relevant to prostate cancer, are summarized below (Table 1).

**Src inhibitors in clinical trials**

Dasatinib (BMS-354825) is an orally available small molecule that inhibits several SFKs (Figure 5) as well as other tyrosine kinases, including Bcr-Abl, Kit, PDGFRβ, and Eph receptors [34, 39, 41, 124, 125]. Dasatinib is approved for chronic myelogenous leukemia (CML) or Philadelphia chromosome-positive acute lymphoblastic leukemia after failure of prior therapy. Dasatinib is active against various human cancers in vitro [41]. In particular, dasatinib suppressed the proliferation of PC-3 human prostate cancer cells [39] and inhibited the adhesion, migration, and invasion of DU145 human prostate cancer cells [34]. Dasatinib also blocked Src and Lyn (an SFK) signaling in DU145 cells, inhibiting downstream phosphorylation of FAK and CAS and reducing activity of matrix metalloprotease 9 [34], a marker associated with the invasion, progression, and metastasis of prostate cancer [126]. In mouse experiments, clinically relevant doses of dasatinib significantly inhibited PC-3 xenograft growth [41]. Furthermore, dasatinib potently inhibited osteoclast proliferation and calcium release in bone resorption assays in vitro and decreased serum calcium levels in rats [127]. A phase II program is ongoing to assess dasatinib in patients with androgen-deprived progressive prostate cancer (NCT00385580) or bone metastasis following breast cancer (NCT00410813) [127]. Phase II trials in CML have demonstrated that dasatinib has limited toxicity [128, 129], allaying concerns that inhibiting multiple signaling pathways might be associated with increased adverse events.

AZD-0530 is an orally active Src/Abl inhibitor in clinical development [130]. AZD-0530 inhibited the growth of PC-3, DU145, LNCaP, and CWR22Rv1 prostate cancer cell lines and suppressed the migration of PC-3 and DU145 cells in vitro [36]. In mouse studies, AZD-0530 inhibited the growth and metastasis of androgen-independent LNCaP cells [42]. AZD-0530 also inhibited osteoclast-mediated bone resorption in vitro [131]. In clinical studies in healthy volunteers, AZD-0530 showed only mild adverse events [132]. Preliminary studies have indicated that AZD-0530 decreased levels of bone

<table>
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<tr>
<th>Agent</th>
<th>Molecular targets</th>
<th>Stage of development</th>
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<tbody>
<tr>
<td>Dasatinib (SPRYCEL®)</td>
<td>SFKs, Abl, Kit, PDGFR, Eph receptors</td>
<td>Approved: CML/Ph+ ALL. Phase II: prostate cancer, breast cancer, hematologic malignancies</td>
</tr>
<tr>
<td>AZD-0530</td>
<td>Src, Abl</td>
<td>Phase II: androgen-independent prostate cancer (planned). Phase I/II: colorectal cancer, pancreatic cancer</td>
</tr>
<tr>
<td>XL999</td>
<td>Src, VEGFR2, PDGFR, Kit, FGFR1</td>
<td>Phase I: lung cancer, renal cell carcinoma, colorectal cancer, ovarian cancer, hematologic malignancies.</td>
</tr>
<tr>
<td>Bosutinib (SKI-606)</td>
<td>SFKs, Abl</td>
<td>Phase I: solid tumors</td>
</tr>
<tr>
<td>PD173955</td>
<td>Src, Yes, Abl, Kit</td>
<td>Phase II: breast cancer, CML/Ph+ ALL. Phase I: solid tumors</td>
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<tr>
<td>CGP76030</td>
<td>SFKs, EGFR, VEGFR, Abl</td>
<td>Preclinical</td>
</tr>
<tr>
<td>CGP77675</td>
<td>SFKs, EGFR, VEGFR, FAK</td>
<td>Preclinical</td>
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<tr>
<td>UC15A</td>
<td>Src</td>
<td>Preclinical</td>
</tr>
<tr>
<td>AP22161</td>
<td>Src</td>
<td>Preclinical</td>
</tr>
<tr>
<td>AP22408, AP23451, AP23588</td>
<td>Src (bone-targeted agents)</td>
<td>Preclinical</td>
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<td>PP2</td>
<td>Src</td>
<td>Research tool</td>
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CML, chronic myelogenous leukemia; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; Ph+ ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia; SFKs, Src family kinases; VEGFR, vascular endothelial growth factor receptor.
Several other SFK inhibitors have shown preclinical activity against prostate cancer cell lines or bone cells, but have not reached clinical development (Table 1). PD173955 is a potent inhibitor of SFKs, Abl, and Kit and has antiproliferative effects on various cancer cell lines, including DU145 prostate cancer cells [40, 136]. PP2, an SFK inhibitor used in research, significantly inhibited DU145, PC-3, and LNCaP cell migration [38] and androgen-independent growth of LNCaP cells in vitro. Two related compounds, CGP76030 and CGP77675, inhibit SFKs and several other kinases [93, 137]. Both agents reduced the proliferation, migration, and adhesion of PC-3 cells, and inhibited osteoclast activity in vitro and in vivo [93, 95]. CGP76030 also decreased breast cancer metastasis to bone in mice and impaired osteoclast-mediated bone resorption [99]. UCS15A [138] and AP22161 [96], blockers of Src protein–protein interactions, inhibited osteoclast bone resorption in vitro. Bone-targeted Src inhibitors have been designed, including AP22408, AP23451, and AP23588. These compounds have demonstrated antistromal and antiresorptive activity in vitro and in vivo [91, 139, 140]. Further developments are awaited to see if any of these agents reach clinical studies.

**conclusions**

Src is the oldest and best studied proto-oncogene. A wide range of evidence indicates that Src and SFK signaling may be important in the oncogenesis of prostate cancer and other tumor types. SFKs represent novel therapeutic targets for advanced prostate cancer, including both androgen-dependent and -independent stages. In vitro data indicate that Src/SFK inhibition may also be of therapeutic use in the context of bone metastases. Of current Src/SFK inhibitors, dasatinib has the broadest range of data supporting a potential efficacy in prostate cancer. Several issues regarding the optimal use of these agents in the clinic still need to be addressed, including optimal dosing, choice of combination treatments, suitable patient groups, and appropriate trial end points. The availability of treatments with novel cellular targets and the potential for combination therapy increase the likelihood of more effective treatments becoming available in prostate cancer in future.

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**references**


