Treatment for Advanced Tumors: Src Reclaims Center Stage

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Background

The non–receptor protein tyrosine, Src, is a 60-kDa protein that is the archetypal member of a nine-gene family, including Src, Yes, Fyn, Lyn, Lck, Hck, Fgr, Blk, and Yrk, that plays a critical role in regulation of proliferation, differentiation, migration, adhesion, invasion, angiogenesis, and immune function (for detailed reviews, see refs. 1–4). All Src family kinases are myristoylated at the NH₂ terminus, and several are additionally palmitoylated downstream of the myristoylation site. These fatty acid moieties are required for localization to cellular membranes. Each Src family kinase contains a poorly conserved (“unique”) domain and three conserved Src homology domains: SH2, SH3, and SH1 or protein tyrosine kinase domain. Critical to the regulation of Src is a COOH-terminal tyrosine (Y530) that, when phosphorylated by C-terminal Src kinase (Csk), leads to a more inactive Src conformation, as has been shown by numerous molecular studies and confirmed by crystal structures (5). An autophosphorylation site (Y418) is indicative of, but not entirely diagnostic for, Src activation. Biochemical and structural studies have discerned the general mechanism by which Src activity is regulated (6).

A myriad of cellular stimuli, including polypeptide growth factors, hormones, integrin aggregation, stress, cell cycle progression, and undoubtedly others, lead to the phosphorylation of signaling proteins for which the Src SH2 domain has higher affinity than for its own COOH-terminal phosphorylated site. Src thus associates with numerous proteins (depending on the input signal) and assumes an active conformation through phosphatase-mediated dephosphorylation of Y530 and autophosphorylation of Y418. Through its SH2 and SH3 domains, Src further associates with structural and signaling proteins, and the resulting complexes are critical to Src’s role in diverse cellular processes, as illustrated in Fig. 1. Specificity of Src signaling stems from the composition of these complexes as well as their duration and subcellular localization and may be additionally influenced by phosphorylation sites on Src mediated by protein kinase C, protein kinase A, cdc2 kinase, and growth factor receptors (7). In turn, Src substrates include signaling enzymes, cytoskeletal proteins, mitotic regulators, etc. (8).

Recent Advances in Roles of Src in Tumor Progression

Recent studies using in vitro and in vivo models provide strong support for the use of Src inhibitors for cancer therapy. In models of epithelial cancer, Src activation promotes a more migratory and invasive phenotype, inducing morphologic and biochemical changes more commonly associated with fibroblasts and cells of mesenchymal origin (17), often termed epithelial to mesenchymal–like transition. This phenotype is associated with increased formation of integrin adhesion structures and decreased E-cadherin–mediated cell-cell contacts (18). Intact Src SH3 and SH2 domains are essential for formation of the focal adhesion–like integrin adhesion complexes (19), and Src catalytic activity is important for the dynamic modulation (including elongation and retraction) that occurs at these sites (20). A current area of intense investigation is proteomic analysis of whether specific alterations (such as phosphorylation) in proteins in these complexes will be predictive markers for patient response to Src inhibitors.

Another key role of Src that may be exploited therapeutically is its regulation of critical angiogenic factors that promote tumor progression. Src activation is required for hypoxia-mediated expression of vascular endothelial growth factor (VEGF; ref. 21), and in mouse models using colon cancer cells, inhibition of Src inhibits VEGF expression and limits tumor growth (22). More recently, we have shown that Src regulates both constitutive and growth factor–induced VEGF and interleukin-8 expression (23, 24) and that Src activation up-regulates VEGF mRNA.
transcription by activation of signal transducers and activators of transcription 3, which forms a complex with hypoxia-inducible factor-1 and other factors on the VEGF promoter (25). Using in vivo models of angiogenesis, we have shown that Src inhibition effectively blocks neovascularization (26), suggesting an anti-angiogenic role for Src inhibitors in the clinic. Equally important to the role of Src in promoting expression of proangiogenic molecules in tumor cells is its function in endothelial cells. Src family members are obligatory intermediates in promoting biological functions of VEGF through VEGF receptors on endothelial cells (27). Src activation leads to increased permeability of endothelial cells, and recent studies using src−/− mice and Src inhibitors in mouse colon cancer models show that abolishing or reducing Src expression/activity decreases tumor cell extravasation, thereby decreasing experimental metastases (28). As in tumor cells, activated Src promotes a mesenchymal-like phenotype in endothelial cells, increasing migratory potential of these cells as well (29). Current results therefore suggest Src activation in tumor cells indirectly regulates Src activity in endothelial cells. Increased Src activity in the tumor cells increases VEGF expression, resulting in increased binding to VEGF receptors on endothelial cells. This process then leads to association of Src with these receptors, increasing Src activity in endothelial cells as well. This continuing cycle promotes Src-mediated increases in migratory potential and permeability of endothelial cells and

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**Fig. 1.** Src-mediated pathways contributing to tumor progression. As discussed in the text, Src family members are obligate intermediates in numerous signaling pathways governing cell proliferation and homeostasis. In tumor cells, association of Src with overexpressed and mutated receptor tyrosine kinases leads to constitutively increased enzymatic activity of Src, thereby activating pro-survival pathways (green) and angiogenic pathways (teal). Aberrant integrin function and/or overexpression of focal adhesion kinase (FAK) result in Src activation in focal adhesion complexes, contributing to cell survival, and activating pathways, leading to increased motility and invasion (red). Although activation of Src by both receptor tyrosine kinases (RTK) and focal adhesion kinase is known to contribute to proliferation of some cell types (primarily through the Ras pathways, yellow), most Src inhibitors have little effect on tumor cell proliferation in vitro or in vivo. It should be noted also that there is considerable "crosstalk" among the pathways, with several of the intermediates key to multiple biologic effects of Src activation. PI3K, phosphatidylinositol 3-kinase; STAT3, signal transducers and activators of transcription 3; IKK, IκB kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; IL-8, interleukin-8; ERK, extracellular signal-regulated kinase; NF-κB, nuclear factor-κB.
facilitates tumor cell extravasation. Inhibitors of Src thus have the potential to interfere with this cycle by affecting biological functions in both tumor and tumor-associated endothelial cells that contribute to metastasis. The importance of targeting tumor-associated endothelial cells for therapeutic efficacy has been highlighted from recent work on inhibitors of receptor tyrosine kinases in clinical trial (30–35); similar principles may apply to Src inhibitors.

Another example of the potential efficacy of Src inhibitors on both tumor and normal cells is in treating bone metastases. In cultured prostate tumor cells, the Src inhibitor, dasatinib, has the expected effects on suppressing tumorigenic properties (36). Src also plays an essential role in normal osteoclast function that is absolutely required for bone resorption, however (37). Src inhibitors, by affecting Src-mediated functions in both tumor cells and osteoclasts, have the potential to interfere with the well-described vicious cycle of tumor cells promoting bone destruction that in turn yields growth factors that promote further tumor growth. Bone-targeted Src inhibitors are being developed to further test this possibility.1 The Src-selective inhibitor, AZD 0530, does indeed affect osteoclast function in humans, inhibiting osteoclastic bone resorption.2

Clinical/Translational Advances

Currently, three inhibitors, dasatinib (BMS354825; Bristol Meyers Squibb Oncology, Princeton, NY), AZD-0530 (AstraZeneca, Macclesfield, United Kingdom), and SKI-606 (Wyeth Research, Pearl River, NY), have reached phase 1 clinical trials. These small molecule inhibitors are directed at the ATP-binding site of Src family kinases and thus are also inhibitors of kinases with closely related structure, such as Abl. Each inhibitor has shown activity against Src in preclinical studies, however. In vitro, dasatinib suppresses invasion and induces cell cycle arrest in head and neck squamous cell carcinomas (38), affects properties of prostate tumor progression (36) and in orthotopic mouse models of pancreatic carcinoma, greatly inhibits the development of liver metastases (39). AZD0530 shows activity at the nanomolar level against v-Src–transformed cells in models of invasion and migration, supporting the hypothesis that Src is an anti-invasive target rather than a direct antiproliferative target. Dramatic inhibition of human breast cancer cell (MDA-MB231) migration by the Src inhibitor AZD0530 has been shown in vitro (40). SKI606 shows activity against colon tumor cells in mouse models (41).

Given that Src is involved in so many fundamental cellular processes, it might be expected that Src-selective inhibitors would be toxic. Knockouts of Src and other family members are viable, however, (42), and current studies on Src/Abl inhibitors in chronic myelogenous leukemia patients (used for their effectiveness against Bcr-Abl) show only limited toxicity. Thus, numerous clinical trials on each of the previously described inhibitors are ongoing or soon to be initiated. A number of different strategies are being used, including trials with a Src inhibitor as single agent in metastatic colon cancer, and in various combinations in other solid tumors. Recent evidence suggests logical combinations of chemotherapeutic agents with Src inhibitors in specific tumors. Some gleevec-resistant chronic myelogenous leukemia patients (43), gemcitabine-resistant pancreatic tumor cells (44), and taxol-resistant ovarian tumor cells (45, 46) have all been associated with increased Src activation, and Src inhibitors sensitize cells to these standard chemotherapeutic agents. As yet, ongoing clinical trials are in too early a stage to determine potential effectiveness of any of the Src inhibitor trials, but in phase I trials, dasatinib seems generally well tolerated as is AZD0530.2

Although the successes of the ongoing clinical trials using these inhibitors remain to be determined, the definition of success needs to be considered carefully. Given the roles for Src in tumor progression and metastasis, the expectations from ongoing and potential future phase II/III trials are likely to differ from many other signal transduction-targeted inhibitors and the efficacy end points for the clinical trials will need to be chosen carefully. End points, such as disease stabilization and time to recurrence, may be more important to assess than more classic measures, such as partial and complete remission. Old lessons will undoubtedly be relearned, including the fallibility of single-agent therapy for most solid tumors. Toxicities of these agents, which still lack complete selectivity, are likely to be problematic in some patients, and drug resistance will almost assuredly arise. Nevertheless, Src-selective agents may advance new concepts in cancer therapy, including how to better limit metastatic spread without eradicating tumor growth, and the importance of targeting the host stromal cells as well as the tumor cells themselves to achieve efficacy. Because of the current flurry of studies with Src inhibitors, these predictions will be tested rapidly. The success of both ongoing laboratory work and clinical trials will determine if Src remains on center stage, stimulates more imaginative approaches to therapy of advanced stage disease, or returns to the wings with respect to its relevance as a therapeutic target for solid tumors.

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