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Src-family tyrosine kinases as therapeutic targets in advanced cancer

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1. ABSTRACT

Src-family tyrosine kinases (SFK) play critical roles in mediating many cellular pathways such as proliferation, adhesion, survival, differentiation and cell motility. There is clear evidence that SFK activity is increased in many human cancers, either through gene amplification, transcriptional upregulation, posttranslational modification by activated upstream growth factor receptors, and even in rare cases, by mutations known to increase intrinsic tyrosine kinase activity in oncoviral forms of SFK. Many recent studies using animal models of human cancer seem to indicate that SFK may only be appropriate therapeutic targets in a subset of primary tumors because of the existence of multiple independent pathways that mediate oncogenic signaling. In contrast, SFK seem to be required for specific parameters of malignant progression, such as recurrence and/or metastasis- especially involving growth in the bone microenvironment. The resulting development of SFK antagonists, and their progression through clinical trials, has brought renewed focus on this tyrosine kinase family as critical mediators of the so-called lethal phenotype of cancer.

2. INTRODUCTION

Src is the prototypic member of a family of non-receptor tyrosine kinases consisting of two subgroups: the ubiquitously expressed Src, Yes and Fyn, versus the kinases expressed in specialized cell types such as hematopoietic (Blk, Fgr, Hck, Lck, Lyn and Yrk) or epithelial (Frk) cells (1,2). The original identification of Src as the oncogene of Rous sarcoma virus indicated that even a single point mutation or the truncation of a C-terminal Tyr-527 residue (Tyr-530 in human Src) in the viral Src allele was sufficient to induce constitutive tyrosine kinase activity when introduced into the so-called cellular-Src proto-oncogene allele (3). Analysis of these mutations subsequently identified several so-called Src-homology (SH) domains: the kinase domain (SH1), a phosphotyrosine-binding domain (SH2) and a PxxPxP-binding domain (SH3) (*4*). In addition to all the SFK encoding these three functional motifs, many other signaling proteins including so-called adaptor proteins encode one or more SH2 and/or SH3 modules that facilitate protein-protein interactions during signal transduction (5). Structural studies of SFK suggest that internal interactions between the SH2/3 domains with their cognate ligands

normally hold the proteins in a "closed", kinase-inactive conformation, whereas the kinase-active conformation can be achieved by mutation at these sites, by interaction of these sites with other signaling proteins, or by specific posttranslational modifications such as autophosphorylation at Y416 (Y419 in human Src)(6,1).

Many studies in the last 20 years have demonstrated upregulated SFK in cancer cell lines and in primary tumors, both at the protein and tyrosine kinase activity level (reviewed in (*7*). Although "viral-Src-like" activating mutations have been identified in small subsets of colon and breast cancers (8,9), the vast majority of cancers involve upregulation of WT-Src. The current belief in the field, therefore, is that genetic mutations and epigenetic changes that accumulate during cancer progression result in overexpressed, activated growth factor or adhesion receptors that directly or indirectly activate SFK and their downstream oncogenic pathways. Indeed, most cancers exhibit increasing activity levels of one or more SFK with progression to more aggressive phenotypes (*7*). Taken together, these data suggest that SFK inhibitors would have their greatest clinical impact in preventing or treating advanced stages of cancer such as recurrence and metastasis. In the paragraphs below, I will review the most recent advances in our understanding of how SFK drive cancer malignancy, the flurry of SFK antagonist development and testing, and the future prospects for targeting SFK in advanced cancers.

3. CRITICAL ROLE FOR SFK IN CANCER MALIGNANCY: PRECLINICAL MODELS

There is a large corpus of data showing that SFK members are required for the proliferation, differentiation, cell motility or survival of many untransformed and cancer cell lines in culture (reviewed in (10,11). With the advent of genetic knockdown technologies, many groups have shown that the loss of specific SFKs has little effect on primary tumor growth in animal models but can prevent parameters of malignant, secondary growth. Below is an incomplete set of examples of such studies. Trevino *et al.* showed that shRNA-mediated Src knockdown had no effect on the incidence of primary orthotopic human pancreatic L3.6pl tumors in nude mice, yet this strongly suppressed the generation of liver metastases (*12*). Although Src knockdown in human FG pancreatic cancer cells partially reduced primary orthotopic tumor growth in nude mice, it severely suppressed the increased spontaneous metastatic potential induced by ectopic alpha(v)beta(3) integrin expression (*13*). Park *et al.* showed similar effects of Src knockdown on the generation of lymph node metastases by orthotopic PC3-LN4 tumors (14). Rucci *et al.* (15) and Myoui *et al.* (16) used kinase-dead versions of Src as a dominant-interfering allele to show that the loss of Src kinase activity suppressed experimental bone metastases formed after intracardiac injection of the human MDA-MB-231 breast cancer cell line. The knockdown of Lyn resulted in a small but significant inhibition of primary Ewing's sarcoma growth but this had even greater effects on the generation of metastatic lesions (17). Lastly, Guo *et al.* demonstrated that the loss of Src-mediated tyrosine phosphorylation of the androgen receptor (resulting from Src shRNA expression) in CWR22R1 human prostate cancer cells prevented tumor recurrence in castrated male mice (18).

One mechanism by which Src seems to inhibit metastatic growth is by suppressing neovascularization at distal sites of tumor cell dissemination. c-Src activation is required to induce vascular endothelial growth factor expression and secretion (19), most probably due to its ability to downregulate HIF-1∀ expression (20), such that Src knockdown suppresses the metastatic potential of human colon cancer cells in mouse models (21). Our lab showed that the SSeCKS/Gravin/AKAP12 gene, an antagonist of Src-mediated oncogenic transformation (22) and podosome/invadosome formation (23), can selectively suppress neovascularization at lung metastatic sites by downregulating VEGF expression (24) through the disengagement of growth factor activation of Src from MAP kinase pathways (25). Several studies from the Cheresh lab argue for the requirement for Src signaling to facilitate activation and recruitment of vascular endothelial cells and other cellular effectors of the tumor microenvironment to peripheral sites of growing metastases (26,27).

Increased Src activation has been reported in cancer cell lines exhibiting resistance to chemotherapeutics (28-34), and thus, groups have focused on preventing resistance by combining SFK inhibitors with standard cytotoxic chemotherapies (30,34-36) or on treating resistant cells with SFK inhibitors (37).

Many cancers, such as breast and prostate cancer and multiple myeloma, metastasize to the bone. The crosstalk of secreted factors between tumor and specific bone cells results in a so-called "vicious cycle" that increases both bone destruction and tumor growth. Metastatic cells interfere with normal bone maintenance and remodeling programs involving a crosstalk between osteoblasts and osteoclasts via the RANKL-osteoprotegerin axis (38,39). Src activity is required for osteoclast activation (40-43) and its inhibition suppresses the formation and growth of bone metastases (16, 44-49). Moreover, in patients with androgen-independent prostate cancer, higher SFK activity levels correlated with increased levels of bone metastases (31). Higher SFK activity levels in primary breast cancer lesions also correlated with increased chances of disease relapse as bone metastases (50). Lastly, there is mounting evidence that insulin-like growth factor (IGF) and IGF-binding proteins facilitate bone development of prostate metastases (51), and thus, Src is thought to be an appropriate target in this context because of its requirement for IGF-1 receptor upregulation by androgens (52).

4. THERAPEUTIC SFK INHIBITORS

A host of small molecule SFK inhibitors are being tested in pre-clinical and clinical trials as monotherapies and in drug combination studies. These include: Dasatinib (BMS-354825; Sprycel[trademark]),

Bosutinib (SKI-606), Saracatinib (AZD0530), PD180970 (53), SU6656 (54,55), KXO1 (KX2-391) (56,57), CGP76030 (58), AP23451 (59), AZM475271 (60-62). Others, such as INNO-406 (NS-187), XL-999 and XL-228, were developed as inhibitors of the Abl non-receptor tyrosine kinase, although they have shown some ability to inhibit SFK (63,64). Dasatinib is the only of these that is approved by the U.S. Food and Drug Administration, and this was for use in chronic myelogenous leukemia or Philadelphia-chromosome-positive acute lymphocytic leukemia. Several recent reviews describe the testing phase and tumor settings for these compounds (65-67). A review of the NCI Clinical Trials Website (http://www.clinicaltrials.gov/ct2/results?term=Src+inhibitors&pg=1), accessed in May 2010, indicates that Dasatinib, Bosutinib and Saracatinib are being tested at the Phase II and III levels in small and non-small cell lung carcinoma, breast, prostate, colorectal, liver, ovarian pancreatic cancer, sarcomas, head and neck squamous cell carcinoma and melanomas, with the remaining SFK inhibitors being tested in Phase II trials on limited tumor settings.

Future directions- A major consideration in the development and testing of SFK-based therapeutics is the ability to identify valid biomarkers of drug efficacy in patients, and additionally, to develop appropriate patient-specific genomic signatures that predict or explain clinical response. One confounding factor in this regard has been the realization that compounds developed initially as Src- or SFK-specific inhibitors subsequently are shown to have a wider range of targets. For example, Dasatinib, originally envisioned as a Src/Abl inhibitor (68), actually targets at least 40 receptor and non-receptor tyrosine kinases including SFK and EGFR (69-71). Thus, given that many human cancer types exhibit activation of multiple, redundant oncogenic pathways (33), it will be difficult in pre-clinical models and in clinical trials to attribute whether anti-tumor efficacy is due to the loss of SFK activation or whether this is a bystander effect. Nonetheless, some groups have already identified potential SFK-related biomarkers for Dasatinib action such as the loss of a shared SFK-poY419 epitope or an epitope (poY845) for the autophosphorylated EGFR (71-73). This raises the issue as to whether a more promiscuous inhibitor might have clinical advantage because of its ability to target multiple oncogenic pathways. Clearly, this will require further study to resolve. However, the advantage for the more specific SFK inhibitors might lie in their lack of toxicity, based on their narrow range of targets. As an example, the widely reported cardiotoxicity induced by Dasatinib is believed to be attributed to its strong inhibition of c-Abl (74), and moreover, Dasatinib has strong immunosuppressive activity (75-77), possibly correlating with its ability to inhibit ZAP-70 (Gelman, I.H., unpublished data). In contrast, KXO1 is a poor inhibitor of Abl and has little effect on ZAP-70 (Gelman, I.H., unpublished data), correlating with no reported cardiotoxicity and little lymphocytopenia in Phase I trials (56). Nevertheless, the clinical side-effects of most SFK inhibitors are manageable, and some are even well tolerated based on Phase I data (56). With the renewed appreciation for SFK as important therapeutic targets in cancer malignancy, and with the growing development of drugs in this sector, there is confidence that SFK inhibitors will offer benefit for many cancer patients, especially those with recurrent and/or metastatic disease. In this regard, there will have to be increased future commitment to clinical studies that include parameters of advanced cancer as therapeutic endpoints rather than just shrinkage of primary tumors.

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