Targeting the Tumor Microenvironment With Src Kinase Inhibition

Alicia S. Chung and Napoleone Ferrara

Although most cancer therapies are directed against tumor cells, an emerging area of cancer therapeutics focuses on targeting cells of the tumor microenvironment. Inhibiting the Src family kinase with dasatinib decreases tumor growth through inhibiting growth of tumor-associated endothelial and myeloid cells.

In this issue of Clinical Cancer Research, Liang and colleagues (1) report that dasatinib, a small molecule inhibitor of multiple kinases including the Src family kinase (SFK) and the fusion oncogene BCR/ABL, affects tumor growth through inhibiting the recruitment and function of stromal cells within the tumor microenvironment.

SFK is composed of nine nonreceptor protein tyrosine kinases including c-Src, Fyn, Yes, Lyn, Lck, Hck, Blk, Fgr, and Yrk. SFKs represent the largest family of nonreceptor tyrosine kinases that can interact directly with receptor tyrosine kinases, G-protein-coupled receptors, steroid receptors, signal transducers and activators of transcription, and molecules involved in cell adhesion and migration. Their aberrant regulation has been implicated in promoting tumor growth and metastasis (2). Moreover, SFK activity has been shown to be important in mediating vascular endothelial growth factor (VEGF)-induced signaling in endothelial cells in response to hypoxia and during angiogenesis (3).

Dasatinib has been shown to be an effective therapeutic for imatinib-resistant chronic myelogenous leukemia. Preclinical data have also shown dasatinib’s inhibitory activity in solid tumors including head and neck squamous carcinoma, pancreatic adenocarcinoma, and prostate cancer cells, suggesting a possible widening in its use outside of leukemia treatment (4). Following identification of additional dasatinib targets including c-kit, platelet-derived growth factor receptor (PDGFR), and EphA2, this compound has also been shown to inhibit PDGFR-β and Src signaling-mediated angiogenesis in multiple myeloma (5).

Tumors are highly complex tissues composed of neoplastic cells surrounded by distinct stromal cell types that together make up the tumor microenvironment. There are both clinical evidence and mouse model studies showing that the interaction between tumor and cells within their microenvironment is important in mediating tumor initiation, progression, and response to anticancer therapy (6). Furthermore, numerous studies have shown that tumor cells can actively recruit stromal cells such as inflammatory cells, vascular cells, and fibroblasts, which together functionally generate the microenvironment that fosters tumor growth.

Here, Liang and colleagues (1) report that dasatinib suppresses tumor growth in vivo by inhibiting the recruitment and function of endothelial and myeloid cells (as defined by Cd11b positivity and Cd11c negativity) and SFK signaling in these cell types. Using primary human umbilical cord endothelial cells (HUVEC), the authors showed that dasatinib treatment inhibits cell migration in response to VEGF and basic fibroblast growth factor (bFGF) and can induce cellular detachment and apoptosis likely via anoikis. In HUVEC cells, dasatinib selectively inhibited Src-dependent phosphorylation of downstream molecules including FAK, p130CAS, and paxillin, which together with SFKs have been suggested to be involved in the regulation of cell adhesion, survival, proliferation, and migration (7). Together these data suggest that dasatinib can modulate HUVEC cell survival and migration via inhibition of VEGF and the bFGF-mediated activation of SFK/FAK/p130CAS/paxillin focal adhesion signaling pathway.

The authors also employed a prostate cancer cell line (DU145) and a colon cancer cell line (Colo205) for their relative resistance to dasatinib in culture. This feature facilitated the testing of these cell lines as xenograft tumors to investigate the effect of dasatinib on the tumor microenvironment. In the Colo205 model, dasatinib treatment inhibited tumor growth by 50% compared with vehicle-treated tumors and potently inhibited SFK phosphorylation in the tumor-associated endothelial cells and tumor cells while having no effect on total SFK protein expression. In both xenograft models, dasatinib inhibited tumor growth, induced tumor cell apoptosis, and reduced tumor microvesSEL density likely via inhibition of SFKs/FAK/p130CAS/paxillin phosphorylation in both endothelial and tumor cells. However, it is not possible to reconcile...
entirely whether the decrease in tumor growth is predominately attributed to dasatinib’s effect on vascular density or its direct effect on tumor cells as dasatinib also potently inhibited SFK signaling in tumor cells.

Because of the known function of tumor-associated myeloid cells in regulating tumor growth, the authors next turned to investigating the effects of dasatinib on this stromal cell type. Compared with vehicle treatment, dasatinib-treated tumors were associated with fewer F4/80+ macrophages and CD11b+ myeloid cells. To further determine whether myeloid cells were targets of dasatinib, CD11b+/CD11c− myeloid cells isolated from the spleens of tumor-bearing mice were tested for SFK, FAK, and paxillin phosphorylation, and were found to be dramatically inhibited in CD11b+/CD11c− myeloid cells from treated mice compared with control. Furthermore, dasatinib inhibited the migratory ability of these cells in response to tumor cell-secreted factors upon treatment ex vivo. These data together suggest that dasatinib may be inhibiting the recruitment of tumor-associated myeloid cells, at least in part through its inhibition of SFK-mediated cell migration (Fig. 1).

Recent studies have implicated Cd11b+ Gr1+ myeloid cells in refractoriness to anti-VEGF therapy in mouse models (8), and Bv8 is up-regulated in these cells by granulocyte colony-stimulating factor (G-CSF). Thus, G-CSF may promote tumor angiogenesis through a Bv8-dependent pathway that bypasses VEGF and renders tumors refractory to anti-VEGF therapy (9). Therefore, it would be of interest to further investigate whether dasatinib also impairs recruitment of Cd11b+ Gr1+ myeloid cells. Furthermore, it is tempting to speculate that combining dasatinib with a VEGF pathway inhibitor might have additive anticancer effects. However, if indeed dasatinib inhibits recruitment and function of Cd11b+ Gr1+ myeloid cells, such a therapy may also negatively affect immune function, as subsets of Cd11b+ Gr1+ myeloid cells have been implicated in suppression of T-cell-mediated immune function, hence the denomination of myeloid-derived suppressor cells (10).

Matrix metalloproteinases (MMP) including MMP-9, which can degrade extracellular matrix components, have emerged as regulators of tumor angiogenesis and progression (11). In the Colo205 xenografts, almost all MMP-9+ cells were CD11b positive, whereas dasatinib treatment led to significant reduction of MMP-9+ and MMP-9+/CD11b+ cells in the tumor microenvironment. The expression of MMP-9 was also decreased upon dasatinib treatment in both tumor cells and tumor-associated CD11b+/CD11c− myeloid cells suggesting that dasatinib not only affects recruitment of tumor-associated myeloid cells but also the expression of a tumor-promoting factor such as MMP-9.

This study presented by Liang and colleagues provides evidence to further support the validity of targeting the tumor microenvironment as a therapeutic approach and also raises many interesting and challenging questions. As their data suggest, SFK activity within endothelial and myeloid cells is likely elicited by tumor-derived external cue(s). What are such tumor-derived factors that may be acting distally in cellular recruitment or locally within the tumor microenvironment? What additional signaling pathways are likely activated by such factors and what are their effects on the tumor microenvironment? These questions warrant further investigations.

As tumor cells undergo numerous genetic, epigenetic, and phenotypic changes throughout disease progression, the cells of the tumor microenvironment are known to be more genetically stable, less likely to acquire drug resistance, and therefore may serve as tractable drug targets. Along these lines, anti-angiogenic drugs have been developed and used in the clinic to target tumor endothelial cells as cancer therapeutics (12). Additional strategies have targeted tumor-associated macrophages in solid tumors and tumor-associated fibroblasts for improving malignant cell sensitivity to chemotherapy and radiotherapy (13, 14). A better understanding of the tumor-stromal interactions should lend to the generation of targeted therapies and also to open up the opportunity of combinatorial therapies against both tumor cells and growth-promoting stromal cells of the tumor microenvironment.

Disclosure of Potential Conflicts of Interest

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