Kinase Inhibitors and Cytotoxic Drug Resistance

Steven Grant1 and Paul Dent2
1Division of Hematology/Oncology, Department of Medicine, and 2Department of Radiation Oncology, Virginia Commonwealth University/Medical College of Virginia, Richmond, Virginia

The Src family consists of a group of nonreceptor tyrosine kinases and includes Src, Lyn, Hck, Yes, and Fyn (1). These kinases are critically involved in diverse cellular processes, including cell survival, proliferation, motility, adhesion, and transformation, among others. Elevated or constitutive activation of Src as well as over-expression are commonly observed in epithelial tumors, most notably in colon and breast cancer, but also occur in other tumor types, including pancreatic cancer (2). Src represents the M, 60,000 protein product of the c-src proto-oncogene, overexpression of which has been associated with tumorigenesis, metastasis, and invasion. In addition to promoting transformation, numerous kinases, including both tyrosine and nontyrosine kinases, have been implicated in the development or preexistence of drug resistance. For example, the constitutively activated Bcr/abl kinase, a mutant form of Abl, a nonreceptor tyrosine kinase closely related to the Src family, has been implicated in the pathogenesis of chronic myelogenous leukemia. In addition to providing chronic myelogenous leukemia progenitors with a survival advantage over their wild-type counterparts, Bcr/abl has been shown to confer leukemic cell resistance to diverse chemotherapeutic agents (3). Links between drug resistance and the activity of various receptor tyrosine kinases (e.g., epidermal growth factor receptor) or serine-threonine kinases (e.g., protein kinase C) have also been described (4, 5). For these reasons, attention has begun to focus on kinase inhibitors not only as antitumor agents in their own right, but also as agents potentially capable of circumventing tumor cell resistance to conventional cytotoxic drugs.

Until now, relatively little information has been available to suggest a specific role for Src inhibitors in this strategy, particularly in epithelial tumor cells. Recently, Donato et al. (6), reported that Src inhibitors such as PP2 (4-amino-5-(4-chlorophenyl)-7-(t-buty1)pyrazolo[3,4-d]pyrimidine) and PD180970 effectively induced apoptosis in chronic myelogenous leukemia cells resistant to the Bcr/abl kinase inhibitor imatinib mesylate (STI571) through a Bcr/abl-independent mechanism characterized by increased activation of the Src family kinase Lyn. These findings raised the theoretical possibility that coadministration of Src inhibitors might overcome imatinib mesylate resistance, at least in certain types of resistant cells. In the current issue, Duxbury et al (7) report that PP2 effectively promotes gemcitabine lethality in pancreatic cancer cell lines exhibiting increased activation of Src and a high degree of resistance to this nucleoside analog. Using both pharmacological (e.g., PP2) as well as molecular tools (e.g., dominant-negative and constitutively active Src constructs), the authors convincingly demonstrate a reciprocal relationship between Src activation and resistance to gemcitabine. Interestingly, Src inactivation was associated with several other perturbations that might plausibly contribute to reversal of resistance in these cells, including reductions in ribonucleotide reductase expression, inactivation of Akt, and diminished activity of E2F-1. Significantly, coadministration of PP2 and gemcitabine resulted in synergistic antitumor effects in a murine orthotopic xenograft model. Collectively, these findings make a strong argument for pursuing a strategy combining gemcitabine with Src inhibitors, at least in pancreatic cancer cells displaying certain forms of resistance to this nucleoside analog.

Because drug resistance is such a highly pleiotropic phenomenon, it is difficult to predict with certainty whether a particular kinase inhibitor will overcome resistance to individual cytotoxic agents. For example, drug resistance may stem from enhanced activation of general efflux mechanism (e.g., those related to P-glycoprotein, multidrug resistance protein, lung resistance-related protein, or breast cancer resistance protein), particularly in the case of natural product cytotoxic agents (8). Alternatively, specific biochemical perturbations may develop or preexist within cells that confer resistance by antagonizing conversion of a cytotoxic drug to its active form (e.g., increased activity of ribonucleotide reductase and/or increased levels of dCTP in the case of certain deoxycytidine analogs; Ref. 9). In addition, it is now well established that generic defects in the apoptotic program (e.g., resulting from increased expression of antiapoptotic proteins such as Bcl-2, Bcl-XL, or Mcl-1) are capable of conferring resistance to a broad range of cytotoxic agents (10). The observation that protein kinase C activation may be involved in P-glycoprotein-mediated as well as other forms of drug resistance has prompted the use of protein kinase C down-regulators/inhibitors such as bryostatin 1 or saffingol in attempts to overcome drug refractoriness (11, 12). Bryostatin 1 and the protein kinase C/Chk-1 kinase inhibitor UCN-01 have also been shown to overcome human leukemic cell resistance to the antimetabolite ara-C (1-ß-d-arabinofuranosylcytosine) conferred by overexpression of the antiapoptotic protein Bcl-2 (13). In addition, the HER family kinase inhibitor CI1033 has been reported to overcome breast cancer resistance protein-mediated resistance to topoisomerase 1 inhibitors in glioblastoma cells (14). Although inhibitors of the receptor tyrosine kinase ERBB1 (epidermal growth factor receptor), such as ZD1839, have been reported to interact synergistically with certain cytotoxic agents (e.g., 5-fluorouracil/cisplatin) in preclinical systems (15), data suggesting that such a strategy can overcome drug resistance are relatively limited to date (16). Significantly, there has been no previous evidence that Src inhibitors could circumvent cytotoxic
drug resistance stemming from increased drug efflux or, for that matter, any other mechanism. In this regard, it is important to note that data from the present study revealed the absence of synergism (and, in fact arguably, antagonistic interactions) between PP2 and agents other than gemcitabine (e.g., 5-fluorouracil); moreover, earlier studies suggested that Src activation could potentiate taxane lethality in transformed gall bladder epithelial cells (17). Such findings suggest that synergism between Src kinase inhibitors and cytotoxic agents, when it occurs, may be highly cell type and drug specific. They also provide a cautionary note arguing against implementation of combination trials involving kinase inhibitors in the absence of compelling preclinical data.

As in the case of other novel reports, the findings by Duxbury et al., raise a number of intriguing questions. Foremost among these is which of the downstream events associated with Src inhibition was most directly responsible for enhanced gemcitabine lethality and circumvention of resistance. For example, given the observed down-regulation of ribonucleotide reductase, it would be useful to know whether PP2 did, in fact, result in increased gemcitabine triphosphate formation and/or increased gemcitabine triphosphate:dCTP ratios. If this were to be the case, it would suggest that the primary role of Src kinase inhibition might lie in overcoming resistance secondary to perturbations in gemcitabine metabolism and/or that of competing naturally occurring nucleosides. Another pertinent question is whether inactivation of Akt, a kinase with diverse antiapoptotic functions (18), contributed to enhanced lethality. In this context, Akt down-regulation has recently been shown to increase apoptosis with delay of G2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. Blood 1995;86:1148–58.

References