Resistance to antineoplastic therapy: The oncogenic tyrosine kinase-Bcl-x_L axis

The discovery two decades ago that the Philadelphia chromosome encodes an oncogenic fusion of Bcr and Abl remains among the most important contributions to our understanding of the process of malignant transformation. We now know that Bcr-Abl is one of more than 30 aberrantly activated tyrosine kinases that are expressed in a variety of tumors. Conventional treatment of the tumors in which these proteins are expressed is usually doomed to failure because the activated tyrosine kinases render the tumor cells stubbornly resistant to apoptosis. In this context, it is notable that Zhao and coworkers have uncovered a novel weapon in the resistance armamentarium of these rogue kinases, the suppression of the inactivating deamidation of Bcl-x_L (this issue of *Cancer Cell*).

Tyrosine kinases normally function as tightly regulated switches in the signal transduction network of the cell. If these switches become stuck in the "on" position, they have the potential to induce oncogenic transformation. Members of both the receptor and nonreceptor families of tyrosine kinases can function in this oncogenic role.

Receptor tyrosine kinases (RTKs), such as EGFR and Erb-B2 (Her-2/Neu), are membrane-spanning proteins that are composed of an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic domain that contains a conserved catalytic kinase core and regulatory sequences. Unstimulated RTKs remain in a monomeric form; ligand binding, however, induces dimerization, which facilitates reciprocal *trans*-phosphorylation of the cytoplasmic domain of each the monomeric components. *trans*-phosphorylation in turn actuates the receptor's catalytic kinase function and creates recruitment sites for downstream signaling targets (Schlessinger, 2000).

There are three general mechanisms by which RTKs can become oncogenic, each of which gives rise to a constitutively active dimeric form. (1) In some cancers, genomic rearrangements occur that generate proteins in which the cytoplasmic domain of a receptor tyrosine kinase is fused to a portion of a second protein that maintains the chimera in a stable dimer. (2) Alternately, as a consequence of gene amplification, certain receptor tyrosine kinases are overexpressed in tumor cells, which leads to spontaneous, ligand-independent dimerization. (3) Finally, some receptor tyrosine kinases acquire point mutations that allow them to dimerize in a ligand-independent manner (Blume-Jensen and Hunter, 2001).

Nonreceptor, or cytoplasmic, tyrosine kinases, such as Abl, Src, and Lck, lack extracellular and transmembrane domains. The mechanisms by which their activity is regulated are varied, and accordingly, so are the mechanisms by which they undergo oncogenic activation. Some are rendered constitutively active by fusion to a dimerizing partner, while others are transformed to oncoproteins when they acquire mutations that disrupt autoinhibitory functions (Blume-Jensen and Hunter, 2001).

Like other dominantly acting oncoproteins, oncogenic tyrosine kinases (OTKs) promote proliferation, invasion, and metastasis. However, a unique characteristic of OTKs is that they tend to render cells extraordinarily resistant to DNA damage-induced apoptosis (Skorski, 2002). This is manifested clinically in the finding that cancers in which OTKs are expressed are usually highly resistant to treatment with conventional antineoplastic agents. Although it is likely that several mechanisms contribute to this resistance, a consistent finding is that oncogenic tyrosine kinases induce markedly increased expression of the antiapoptotic protein Bcl-x_L, and that the increased expression is an important component of the resistance to treatment.

A role for Bcl-x_L was first suggested when it was found that expression of Erb-B2 (Her-2/Neu) in an estrogen receptor (ER)-positive breast cancer cell line increased the cellular level of Bcl-x_L and rendered the cells resistant to tamoxifen-induced apoptosis (Kumar et al., 1996). This was an intriguing finding, because high-level expression of Erb-B2 in ER-positive breast cancer is associated with resistance to endocrine therapy. A more direct demonstration of the role of the increased expression of Bcl-x_L in OTK-induced resistance was provided by a subsequent study in which it was found that Bcr-Abl expression in a promyelocytic cell line resulted in increased expression of Bcl-x_L and resistance to a variety of apoptosis-inducing agents and that inhibition of Bcl-x_L expression with antisense restored susceptibility (Amarante-Mendes et al., 1998). Several other potentially oncogenic tyrosine kinases also upregulate Bcl-x_L expression, including EGFR (Karni et al., 1999), c-Src (Karni et al., 1999), and ALK (Zamo et al., 2002). Finally, the Bcr-Abl tyrosine kinase inhibitor STI 571 is effective in killing tumor cells at least in part because it downregulates cellular Bcl-x_L levels (Horita et al., 2000; Oetzel et al., 2000). When considered together, these findings clearly implicate the antiapoptotic activity of Bcl-x_L as an important component of the treatment resistance of tumors that express OTKs.

Now, a new dimension has been added to our understanding of this resistance. In this issue of *Cancer Cell*, Zhao and coworkers present the findings of studies in which they used a transgenic mouse model of T cell lymphoma to dissect the components of the transforming function of an activated form of the Src-related tyrosine kinase Lck (Zhao et al., 2004). In one study, they compared the effects of two different levels of Lck activity on the response to γ radiation and etoposide. Remarkably, the authors found that even though the p53 signaling pathway was equally responsive to treatment in thymocytes expressing either "intermediate activity" (higher activity than wild-type Lck, but nononcogenic) or "hyperactive" (oncogenic) Lck, and both levels of Lck activity induced expression of similar levels of Bcl-x_L, the "hyperactive" Lck was strikingly more effective in preventing both γ radiation-induced and etoposide-induced apoptosis. When the authors set out to define the mechanism underlying this difference, they found that whereas the "intermediate activity" Lck had no effect on γ radiation-induced and etoposide-induced deamidation of Bcl-x_L, Bcl-x_L deamidation was completely

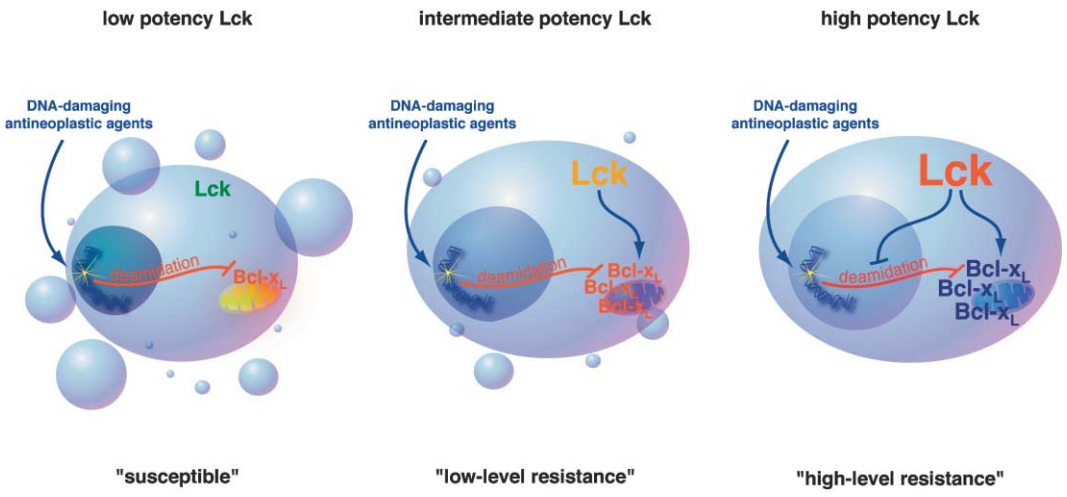

Figure 1. Model for oncogenic Lck-induced resistance to apoptosis

When Lck activity is low (normal), Bcl-x_L is at a basal level and susceptible to deamidation, which leaves the cell susceptible to DNA-damaging agent-induced apoptosis. If Lck activity is increased, but below an oncogenic level, the level of Bcl-x_L is increased, but the Bcl-x_L remains susceptible to deamidation, so the cell displays only a low level of resistance to DNA-damaging agent-induced apoptosis. If Lck activity exceeds the oncogenic threshold, the level of Bcl-x_L is increased and deamidation of the Bcl-x_L is suppressed, which renders the cell highly resistant to DNA-damaging agent-induced apoptosis. It is important to note that it is likely that other effects of increased Lck activity also contribute to resistance.

suppressed by the "hyperactive" Lck (Figure 1).

Deamidation is a posttranslational modification in which an asparagine is converted into an aspartate. It was recently found that Bcl-x_L undergoes deamidation at two asparagines in response to treatment with antineoplastic agents in a variety of tumor cells and that the inhibition of Bcl-x_L deamidation increases the cells' resistance to the effects of these agents (Deverman et al., 2002). This implies that deamidation decreases the antiapoptotic activity of Bcl-x_L. Consistent with this is the finding in the current study that deamidation disrupts the ability of Bcl-x_L to bind to the proapoptotic Bcl-2 family member Bim. It had previously been shown that the antiapoptotic activity of Bcl-x_L is dependent upon its ability to bind to Bim and related proteins (Cheng et al., 2001). Therefore, Zhao and coworkers have provided further evidence that deamidation serves to inactivate Bcl-x_L.

The finding that oncogenic Lck both suppresses Bcl-x_L deamidation and renders the cell resistant to apoptosis raises the possibility that suppression of Bcl-x_L deamidation contributes to the resistance that is characteristic of OTK-induced tumors. This is supported by the finding that deamidation of Bcl-x_L is suppressed in hepatoccellular carcinoma—a tumor in which OTKs are expressed that is notoriously difficult to treat (Takehara and Takahashi, 2003). These are exciting findings because they suggest that stimulation of Bcl-x_L deamidation will at least in part overcome OTK-induced resistance. Thus, a new avenue for treatment has been opened. The challenge now is to delineate the mechanism by which deamidation of Bcl-x_L is regulated and to determine how it is suppressed by OTKs.

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