

A continuous cycle of cell birth and death occurs in essentially all tissues with self-renewal capacity, with new cells being produced through cell division and older cells dying principally through programmed cell death and its morphological counterpart, apoptosis. This delicate balance between cell production and cell death ensures that overall numbers of cells are maintained within physiologically appropriate ranges. Consequently, disturbances in these homeostatic mechanisms can contribute to the origins of cancer, imparting neoplastic cells with a selective growth advantage because of either accelerated rates of cell proliferation, decreased rates of cell demise, or both. Moreover, defects in the cell death pathway can promote chemo- and radioresistance by allowing tumor cells to survive, despite having been damaged by drugs or radiation (1).

In the breast, the numbers of mammary epithelial cells expand and contract in response to estrogens and other hormones. In fact, one of the classical examples of programmed cell death is involution of the postlactating mammary gland, where the loss of lactogenic hormones results in collapse of the gland in large part because of elimination by apoptosis of differentiated mammary epithelial cells. In recent years, it has become increasingly clear that defective programmed cell death mechanisms contribute significantly to the origins and progression of breast cancer. In this issue of *The Journal*, Bargou et al. provide further evidence of this by showing that growth of human breast cancer cell lines in vivo as tumors in immunocompromised (SCID) mice can be suppressed by gene transfer-mediated overexpression of the pro-apoptotic protein Bax (2).

Bax is a member of the Bcl-2 family of apoptosis-regulating proteins whose expression is induced in a p53-dependent manner in some types of cells by  $\gamma$ -radiation, chemotherapeutic drugs, and other forms of genotoxic stress (3). Bcl-2 family proteins appear to regulate a distal step in an evolutionarily conserved pathway for physiological cell death and apoptosis, with some members functioning as suppressors of apoptosis and others as promoters of cell death (reviewed in reference 1). The relative ratios of these various pro- and anti-apoptotic members of the Bcl-2 family have been shown to determine the ultimate sensitivity or resistance of cells to diverse apoptotic stimuli, including chemotherapeutic drugs and radiation, growth factor deprivation, loss of cell attachment to extracellular matrix proteins (an issue of potential relevance to mechanisms of tumor metastasis), hypoxia (a common occurrence in the centers of large tumors), and lysis by cytolytic T cells (reviewed in references 1, 4).

Several Bcl-2 family proteins are expressed in normal mammary epithelium, including the anti-apoptotic proteins Bcl-2, Bcl-X, and Mcl-1, and the pro-apoptotic protein Bax (5). Expression of Bcl-2 in breast cancer cell lines has been shown to be dependent on estrogen, whereas Bax is not (see reference 6 for example). Indeed, several studies have documented strong correlations between estrogen receptor (ER) positivity

and Bcl-2 immunostaining in primary adenocarcinomas of the breast (reviewed in reference 7). Furthermore, consistent with the generally more aggressive nature of tumors which have progressed to an ER-negative, hormone-independent state, patients with ER+, Bcl-2-positive tumors typically enjoy longer disease-free and overall survivals.

These observations on Bcl-2 expression in breast cancers have created a conundrum. If Bcl-2 is an anti-apoptotic protein that has been demonstrated to block apoptosis induction by chemotherapeutic drugs, etc., then why is loss of Bcl-2 expression during progression of mammary carcinomas associated with favorable outcome? A potential answer to this question came last year when two groups reported marked reductions in the expression of Bax in breast cancers. Bargou et al., showed that Bax mRNA levels were reduced compared to normal breast tissue in 10 of 10 breast tumor specimens, whereas levels of mRNA for the anti-apoptotic proteins Bcl-2 and Bcl-X<sub>L</sub> were similar in tumor and normal breast tissue (8). In their present study, these investigators extend this analysis, showing that Bax mRNA levels are reduced in 35 of 36 malignant breast cancer specimens and 4 of 4 carcinoma in situ lesions and demonstrating that gene transfer-mediated restoration of Bax protein in 2 breast cancer lines promotes apoptosis induced by growth factor deprivation (2). Similarly, Krajewski et al., using immunostaining techniques to evaluate Bax protein levels in primary tumors derived from 119 women with metastatic breast cancer, demonstrated heterogeneous percentages of Bax-immunopositive malignant cells among tumor specimens, with 34% of cases having < 10% Bax-positive cells. The subgroup of patients with reduced Bax tended not to respond to therapy (21 vs. 43%;  $P < 0.02$ ) and generally experienced shorter times to tumor progression (median 3.7 vs. 9.0 mo) and shorter overall survival (10.7 vs. 17.1 mo), which were statistically significant in both univariate and multivariate analyses (9). Interestingly, a strong positive correlation was found ( $P = 0.005$ ) between the percentages of Bcl-2 and Bax-immunopositive tumor cells, implying that the same tumor cells that had lost Bcl-2 expression were also the ones with reduced Bax levels.

The notion therefore is that while Bcl-2 levels tend to decline during progression of breast cancers, so too do the levels of Bax. As depicted in Fig. 1, since it is the ratio of Bcl-2 to Bax that determines sensitivity to apoptosis, rather than the absolute levels of either protein, these findings suggest that advanced breast cancers with reduced Bcl-2 are probably no more sensitive to apoptosis than their Bcl-2-positive, less aggressive counterparts and indeed may be more resistant to apoptosis when one considers that other anti-apoptotic Bcl-2 family proteins including Bcl-X<sub>L</sub> and Mcl-1 are usually present in these tumors.

Still, there are many unresolved questions about the molecular mechanisms that regulate Bax expression in breast cancers and the role of Bax in influencing the biology and chemoresponses of these tumors. What, for example, accounts for the reductions in Bax expression in breast cancers and are these changes permanent or alternatively can something be done to restore production of this pro-apoptotic protein? At what point in tumor progression does Bax become reduced? Bargou

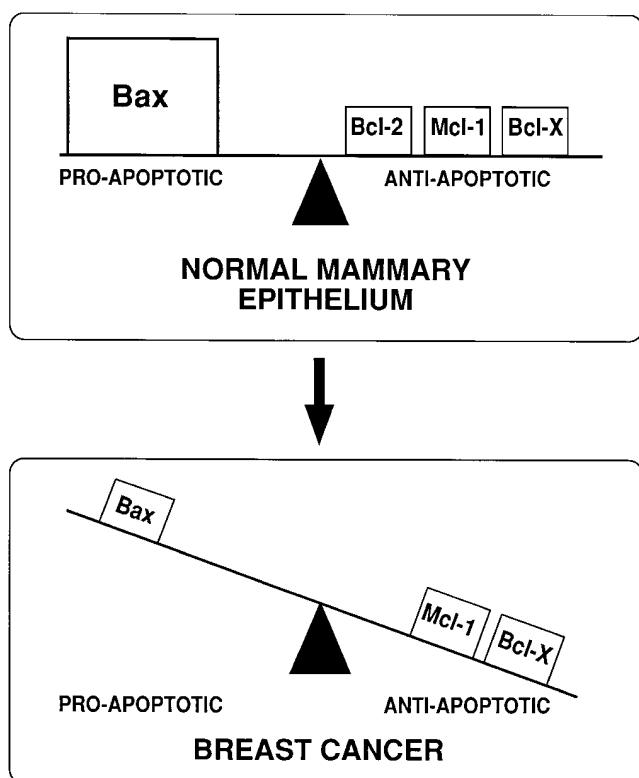


Figure 1. An appropriate balance of pro-apoptotic (*Bax*) and anti-apoptotic (*Bcl-2*, *Bcl-X<sub>L</sub>*, *Mcl-1*) Bcl-2 family proteins is present in normal mammary epithelium (top). During the pathogenesis of adenocarcinomas of the breast (bottom), *Bcl-2* expression is sometimes lost in combination with reduced levels of *Bax*. Since other anti-apoptotic proteins such as *Bcl-X<sub>L</sub>* and *Mcl-1* may still be present, the balance between pro- and anti-apoptotic proteins is hypothesized to be upset in favor of the cell death blockers.

et al. noted reduced *Bax* expression in carcinoma in situ, whereas Krajewski et al. reported retention of *Bax* immunostaining in the in situ component of breast tumors (2, 9). To what extent does *Bax* provide clinicians with a novel prognostic marker for breast cancer patients? So far, *Bax* expression has not correlated with ER, PR, p53, c-erbB2, histological-grade, node-status, or other clinical and laboratory variables,

but *Bax* is positively correlated with *Bcl-2* (9). How can knowledge of the status of *Bax* and *Bcl-2* expression be intelligently used for making clinical decisions about treatment? In the report by Krajewski et al., there was a suggestion that women with *Bax*-negative tumors might benefit from more aggressive therapy (9). Clearly, the answers to these and other questions will require further basic studies, as well as comparisons of *Bax* and *Bcl-2* with other potential prognostic markers in large, well-controlled studies of patients with both node-negative and node-positive disease. In particular, given the probable role of *Bcl-2* family proteins as determinants of chemoresponses, it will be of interest to explore the relation of *Bax* and *Bcl-2* to response and outcome in trials focused on the issue of dose intensification in women with metastatic adenocarcinoma of the breast.

John C. Reed  
Scientific Director  
The Burnham Institute

## References

1. Reed, J.C. 1995. *Bcl-2*: Prevention of apoptosis as a mechanism of drug resistance. *Hematol. Oncol. Clin. of N. Am.* 9:451-474.
2. Bargou, R.C., C. Wagener, K. Bommert, M.Y. Mapara, P.T. Daniel, W. Arnold, M. Dietel, H. Guski, A. Feller, H.D. Royer, and B. Dörken. 1995. Overexpression of the death-promoting gene *bax-a* which is downregulated in breast cancer restores sensitivity to different apoptotic stimuli and reduces tumor growth in SCID mice. *J. Clin. Invest.* 97:2651-2659.
3. Zhan, Q., S. Fan, I. Bae, C. Guillouf, D.A. Liebermann, P.M. O'Connor, and A.J. Fornace Jr.. 1994. Induction of *bax* by genotoxic stress in human cells correlates with normal p53 status and apoptosis. *Oncogene.* 9:3743-3751.
4. Reed, J.C. 1994. *Bcl-2* and the regulation of programmed cell death. *J. Cell Biol.* 124:1-6.
5. Krajewski, S., M. Krajewska, A. Shabaik, T. Miyashita, H.-G. Wang, and J.C. Reed. 1994. Immunohistochemical determination of in vivo distribution of *bax*, a dominant inhibitor of *bcl-2*. *Am. J. Pathol.* 145:1323-1333.
6. Teixeira, C., J.C. Reed, and M.A.C. Pratt. 1995. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving *Bcl-2* protooncogene expression in human breast cancer cells. *Cancer Res.* 55:3902-3907.
7. Lu, Q.-L., P. Abel, C.S. Foster, and E.-N. Lalani. 1996. *bcl-2*: role in epithelial differentiation and oncogenesis. *Hum. Pathol.* 27:102-110.
8. Bargou, R.C., P.T. Daniel, M.Y. Mapara, K. Bommert, K. Wagenert, C. Wagener, B. Kallinich, H.D. Royer, and B. Dörken. 1995. Expression of the *bcl-2* gene family in normal and malignant breast tissue: low *bax-α* expression in tumor cells correlates with resistance towards apoptosis. *Int. J. Cancer.* 60: 854-859.
9. Krajewski, S., C. Blomqvist, K. Franssila, M. Krajewska, V.-M. Wase-nius, E. Niskanen, and J.C. Reed. 1995. Reduced expression of pro-apoptotic gene *Bax* is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. *Cancer Res.* 55:4471-4478.