Letters to the Editor


Letter

Krajewski et al. (1) have recently analyzed the relationship between p53 immunoreactivity and the level of Bcl-2 and Bax in breast cancers using immunohistochemistry, because recent data indicate that wild-type p53 can transcriptionally repress Bcl-2 expression while inducing the expression of Bax. Absence of p53 in knockout mice was associated with increased Bcl-2 and decreased Bax in some tissues (2). When the percentage of p53-immunopositive tumor cells was compared with the percentage of Bcl-2- and Bax-immunopositive cells (as a continuous variable) in breast adenocarcinoma, a significant inverse correlation between Bcl-2 and p53 was found (P < 0.001).

Using the same techniques, we previously compared p53, Bcl-2, and Bax immunoreactivity in neuroendocrine lung tumors, which comprise a spectrum of four entities of increasing grade of malignancy from low grade (typical carcinoids) to intermediate grade (atypical carcinoids) to high grade (large cell neuroendocrine carcinoma and SCLC) (3).

Using three p53 antibodies, including PAB 1801, used by Krajewski et al. (1), and Bcl-2 and Bax antibodies recognizing the 26-kb isoform of Bcl-2 and the 21-kDa isoform of Bax, respectively, as referred to immunoblots, we could demonstrate a direct correlation between p53 immunopositivity and Bcl-2 level of expression (P = 0.005) and an inverse correlation between p53 immunopositivity and Bax level of expression (P = 0.01). Although we did not use comparison of continuous variable but a cutoff at 20% of positive cells for p53 immunopositivity, and scores of Bax and Bcl-2 immunostaining were calculated by multiplying the percentage of positive cells (1–100) by the intensity of staining (1–3), the results on neuroendocrine tumors differed strikingly from those of Krajewski et al. (1). We assumed from previous results that more than 20% of positive p53 cells indicated mutant phenotype for p53. Low-grade neuroendocrine tumors such as carcinoids were characterized by a wild-type p53 (p53 negative), an intense and frequent expression of Bax in 95% of the cases, and a rare overexpression of Bcl-2 in 15% of the cases. In contrast, in high-grade neuroendocrine lung tumors, p53 was immunoreactive in 50% of the cases, whereas Bax expression was observed in 44% of the cases, and Bcl-2 overexpression was intense in 90% of the cases. Despite the differences in the methodology of interpretation of immunostaining, visual inspection of the referenced author’s data allowed comparison between the distribution of the same markers in neuroendocrine lung tumors and breast cancers. It can be concluded that the relationship between p53 and Bcl-2 and Bax level is inverse in breast adenocarcinoma and neuroendocrine lung tumors. This is consistent with the idea that p53 is not the unique factor that contributes to the regulation of Bax and Bcl-2 genes and that this regulation is tissue dependent.

Moreover, when the relationship of Bax and Bcl-2 levels of expression was examined in individual tumors, the levels were found to be directly correlated in breast adenocarcinoma (P = 0.02), suggesting that the expression of these genes may be coregulated to some extent in these breast cancers. In contrast, they were inversely correlated in neuroendocrine lung tumors of any grade (P < 0.0001). Whereas Bax and Bcl-2 have a reciprocal pattern of production in some tissues with glandular differentiation, such as salivary glands and colon (4) as well as in neuroendocrine lung tumors (2), they are found to be in direct correlation in breast adenocarcinoma (1).

Finally, when the prognostic significance of Bcl-2 and Bax was investigated, Bcl-2>Bax was slightly associated with longer survival in node-positive breast adenocarcinoma and highly correlated with shorter survival in operable neuroendocrine lung tumors. We therefore questioned the role of apoptosis deregulation using terminal deoxynucleotidyl transferase-mediated nick end labeling analysis in the prognostic differences between high-grade and low-grade neuroendocrine lung tumors with reverse Bax/Bcl-2 phenotypes. In both high-grade tumors, neuroendocrine carcinoma and SCLC, the apoptotic index was related to the level of Bax and to the Bcl-2:Bax ratio (P = 0.02), but not to that of Bcl-2. However, the apoptotic index was strikingly low (<0.1%) in SCLC where Bcl-2 is quite constantly high, but because neither apoptotic indexes nor Bcl-2 levels were variable in this tumor type, no statistical correlation could be demonstrated to link them. To some extent, at least, this is in a direct line with the finding that Bcl-2 overexpression is related to low apoptosis, which was also demonstrated previously in breast cancer (5) where high Bcl-2 expression was strongly associated with both apoptosis loss (P < 0.0001) and the presence of lymph node metastasis. This is consistent with the expected role of Bcl-2 in cell survival. Neither in breast cancer nor in neuroendocrine lung tumors, however, was p53 directly correlated with apoptosis magnitude. Bax and Bcl-2 thus seem to be more direct downstream regulators of cell death than p53.

This reverse image of Bcl-2/Bax phenotypes and of their prognostic relevance in two different tumor types and tissues reflects the exquisite selectivity and tissue specificity of the functions of Bax and Bcl-2 in tumor growth regulation and the variable role of p53 mutation in the Bcl-2:Bax imbalance in different tissues. Accordingly, although Bcl-2 expression and its p53 partial dependence could be demonstrated in neuroendocrine lung tumors, an inverse correlation between Bcl-2 and p53 immunostaining was found in non-SCLC with no neuroendocrine differentiation (6, 7). Moreover, survival probability was higher in patients who expressed Bcl-2 protein in tumor cells, which is in striking contrast with the low survival probability conferred by Bcl-2 overexpression in neuroendocrine lung carcinoma (2). This recalls a possible role of Bcl-2 in proliferation and cell cycle entry. In stimu-
lated B and T lymphocytes and in colon carcinoma cells, Bcl-2 transgene reduces proliferation and delays cell cycle entry, and these two functions of Bcl-2 were demonstrated to be independent and antagonized by Bax (8). The influence of Bcl-2 on survival probability in breast cancer and non-SCLC suggests that Bcl-2 in these cancers not only reduces the level of apoptosis but also represses cell growth. In some tissues, regulation of cell survival could be coupled to control of cell growth by Bcl-2.

This suggests that Bcl-2 and Bax function and their regulation by p53 could be very selective and highly dependent on differentiation type. Bcl-2 and Bax may play concordant roles in apoptosis in different tissues, but the regulation of cell survival could be differently coupled to control of cell growth in different tumor and tissue types and in neuroendocrine and non-neuroendocrine cells. Only one member of the Bcl-2 protein family may not be sufficient to understand the dysregulation during oncogenesis.

Elisabeth Brambilla
Adrien Negoeescu
Groupe de Recherche sur le Cancer Bronchique
CIF INSERM 97.01
CHU de Grenoble
Hospital Michallon, B. P. 217
38043 Grenoble cedex 09
France

References

Reply

Drs. Brambilla and Negoeescu (1) have drawn a nice comparison of the differences that can be seen in various types of tumors, with regard to the relationship between p53 status and expression of the apoptosis-regulating proteins Bcl-2 and Bax. The rationale for examining correlations between p53 and the Bcl-2 and Bax proteins can be attributed at least in part to our previous studies that demonstrated that wild-type p53 can repress at a transcriptional level the expression of Bcl-2 and induce expression of Bax in some types of cells and tumor cell lines (2–5). However, as we and others have emphasized in past publications (2, 4–7), p53 represents only one of the transcriptional inputs into the promoters of the Bcl-2 and Bax genes. Moreover, the relative amounts of the Bcl-2 and Bax proteins can be regulated through posttranscriptional mechanisms, including mRNA translation and protein turnover (8–10). In the study of SCLCs by Drs. Brambilla and Negoeescu, p53 immunostaining (usually an indication of mutant and hence inactive p53 protein) was correlated with higher Bcl-2 and lower Bax protein immunostaining (11). Thus, the findings in SCLCs support the idea that p53 can be a direct repressor of Bcl-2 and a transactivator of Bax gene expression. However, in many other types of cancer, including adenocarcinoma of the breast, either no relationship between p53 and expression of Bcl-2 and Bax, as defined by immunohistochemical methods, is observed, or the relationships run contrary to expectations (12, 13). Again, this result most likely speaks to the molecular complexity of the regulation of Bcl-2 and Bax protein levels and the importance of cellular context.

In their letter (1), Drs. Brambilla and Negoeescu also reiterate several of the points that we have made in previous publications about the potential interpretations of correlations of Bcl-2 and Bax immunostaining results with clinical outcome for patients with cancer. These issues include: (a) recognition that the ratios of antiapoptotic:proapoptotic members of the Bcl-2 family rather than either alone dictate the ultimate sensitivity or resistance of tumor cells to apoptotic stimuli; (b) realization that Bcl-2 and Bax are only 2 members of a family of at least 14 homologous apoptosis-regulating proteins (and that little is known about the expression of these other Bcl-2 family proteins in cancers); and (c) consideration of recent information that demonstrates that the antiapoptotic proteins Bcl-2 and Bcl-XL not only block cell death but can also inhibit the entry of quiescent cells into the cell cycle, thus impairing proliferation (reviewed in Refs. 14–16).

It should also be noted that studies of archival tumor material by immunohistochemical methods provide only a snapshot in time and do not address the dynamic regulation of Bcl-2 and Bax expression that is likely to occur in tumors. For example, no studies have, to date, explored the relationship between p53 and expression of Bcl-2 and Bax in patients immediately after therapy, when genotoxic stress would be expected to trigger an accumulation of wild-type p53 protein.
Analysis of Bax and Bcl-2 expression in p53-immunopositive breast cancers.

E Brambilla and A Negoescu


Updated version Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/3/11/2181.citation

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.