Expression of bcl-2 in Small Cell Lung Carcinoma Cells

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Abstract

We have investigated the expression of bcl-2 protein in a panel of small cell lung carcinoma (SCLC) cell lines. Five of six cell lines examined expressed relatively high levels of bcl-2 protein and transcripts. The bcl-2 expression in SCLC cells, however, was not always associated with myc expression. Since dysregulation of bcl-2 may be involved in the course of tumorigenesis and/or in the acquisition of drug resistance of tumor cells, the expression of bcl-2 in SCLC cells may become an important indicator in the prognosis or treatment of SCLC.

Introduction

Lung cancer has now become the leading cause of cancer deaths in both men and women in the United States (1). SCLC is an aggressive and highly metastatic disease, and it accounts for about one-quarter of all lung cancer cases. Although SCLC tumors are initially responsive to chemotherapy, multidrug resistance tends to develop after relapse, and the majority of SCLC patients die of their disease within 2 years (1). Cytogenetic and molecular genetic analyses of SCLC have revealed that SCLC cells carry a number of chromosomal abnormalities. Double minutes or homogeneous staining regions, indicative of gene amplification, are often found associated with SCLC. Chromosomal alterations and losses, indicative of gene inactivation, at 1p, 3p, 5q, 9p, 13q, and 17p regions are also common in SCLC (2, 3). In those SCLC cell lines with double minutes or homogenous staining regions, the myc family protooncogenes are often amplified, resulting in overexpression of myc family genes at RNA and protein levels (4, 5). In some cases, the myc family genes are overexpressed even in those SCLC cells without gene amplification (4–7).

The bcl-2 gene is categorically distinct from classical oncopgenic. High levels of bcl-2 expression prevent cell death from a wide variety of cell stresses and cytotoxic chemicals including heat shock (8, 9), ionizing radiation, excess calcium influx (10, 11), and a range of chemotherapeutic drugs (8, 9, 12). These various stress conditions are known to induce a particular type of cell death, called apoptosis, and the bcl-2 protein seems to function as a negative regulator at a certain point of a biological cascade leading to physiological cell death, or apoptosis (13). It has been shown that bcl-2 cooperates with c-myc in cell transformation (14). Recently, it was also found that high levels of c-myc expression, when combined with a block of cell proliferation, induce apoptotic cell death (15, 16). Furthermore, this c-myc induced-cell death is inhibited by the coexpression of bcl-2 (17, 18). Thus, it appears that c-myc and bcl-2 cooperate in cell transformation in such a way that bcl-2 suppresses the cell death promoting function of c-myc. By analogy, N-myc and L-myc might have similar cell-death-promoting activities that are inhibited by the coexpression of bcl-2.

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Materials and Methods

Cell Lines and Culture. All the cell lines used in this study were maintained in RPMI 1640 supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 5% fetal bovine serum, 0.1% ITS (Collaborative Research, Bedford, MA), and 1% OPI (19).

Antibodies. The pan-myc reactive monoclonal antibody, NCM II 143, was produced and characterized in our laboratory (20). The human bcl-2-specific monoclonal antibody, 100, was a gift of Dr. David Mason of John Radcliffe Hospital, Headington, United Kingdom (21).

Immunoblotting. Immunoblotting was performed according to the method previously described (22) except the ECL chemiluminescent detection system (Amersham) was used. Cell extracts were made in the 2D gel sample buffer (9 m urea, 2% Nonidet P-40, 2% mercaptoethanol, and 0.32% 2D Pharmalyte, pH3–10), and the protein content of the samples was determined by the Bio-Rad protein assay kit using bovine serum albumin as a standard and the sample buffer as the blank.

Northern Blotting. Total cellular RNA was prepared from the cell by the method described by Auffray and Rougeon (23). An aliquot equivalent to ten μg of each RNA preparation was subjected to Northern blotting procedure as described by Krozek and Siebert (24). The blot was hybridized with a human bcl-2 probe, Probe A, (25) that had been labeled with 32P. After the blot was washed in 0.1 × SSC containing 0.1% sodium dodecyl sulfate at 65°C, the blot was exposed to a X-ray film at ~70°C for 3 days.

Results and Discussion

bcl-2 Protein Is Expressed at Relatively High Levels in Five of Six SCLC Cell Lines. Expression of the bcl-2 protein in SCLC cell lines was examined by immunoblotting assay using the human bcl-2-specific monoclonal antibody, 100 (21). As shown in Fig. 1, five of six SCLC cell lines (NCI-H69, 209, 345, 378, and 510; but not NCI-H682) expressed relatively high levels of bcl-2 protein. Levels of bcl-2 expression in these SCLC cells were equivalent to or even higher than that of a preB leukemia cell line, 697, and a neuroblastoma cell line, SMS-KCNR. These two cell lines have been known to express relatively high levels of the bcl-2 protein (26, 27). Another neuroblastoma cell line, CHP126, which expresses a low level of the bcl-2 protein, was also included in this analysis as a comparison.

bcl-2 Protein Expression Correlates with the bcl-2 RNA Expression in SCLC Cells. We next examined steady state levels of the bcl-2 transcript in SCLC cells to examine if the bcl-2 protein expression correlates with the expression of bcl-2 transcript. Total cellular RNA was prepared from these six SCLC and SMS-KCNR neuroblastoma cell lines, and the RNA preparations were then subjected to RNA blotting analysis using a human bcl-2 clone, Probe A (25), as a probe. The expression of bcl-2 transcripts in these SCLC lines correlated with the bcl-2 protein expression in these cells. As shown in the Fig. 2, NCI-H69, 209, 345, 378, 510, and KCNR cells express readily

\(^1\) The abbreviation used is: SCLC, small cell lung carcinoma.

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\(^3\) N. Ikegaki, unpublished data.
protein/sample) made from the cell lines indicated were subjected to immunoblotting assay using the human bcl-2-specific monoclonal antibody, 100.

Fig. 1. Expression of bcl-2 protein in SCLC cell lines. Total cellular extracts (5 μg protein/sample) made from cell lines indicated were subjected to immunoblotting assay using the human bcl-2-specific monoclonal antibody, 100.

Expression of bcl-2 in SCLC Cells Is Not Always Associated with myc Protein Expression. The bcl-2 and myc genes have been shown to cooperate in cell transformation in such a way that bcl-2 suppresses the cell-death-promoting function of c-myc (14, 17, 18). Thus, it was of interest to examine if there is any association between bcl-2 and myc expression in SCLC cells. The expression of the myc protein was examined by immunoblotting assay using the pan-myc reactive monoclonal antibody, NCM II 143, which detects three members of the myc family of proteins including the short form of L-myc (4). As shown in Fig. 3, five of six SCLC cell lines express one of the myc family proteins. NCI-H69 expresses the M, 60,000/63,000 N-myc protein at relatively high levels as do two neuroblastoma cell lines, CHP126 and KCNR. NCI-H82 expresses the M, 58,000/64,000 c-myc protein at relatively high levels as does the 697 preB leukemia cell line. NCI-H209, 378, and 510 express the M, 60,000/63,000/66,000 L-myc protein, though there are variations in the level of M, 66,000 species in these cell lines (i.e., readily detectable in 510, a minimal level in 209, and none detectable in 378). Only NCI-H510 expresses detectable levels of the short form of the L-myc protein. NCI-H345 expresses no detectable myc protein, although it expresses low levels of the L-myc transcript.5 It should be noted that these observations on the expression of myc proteins in SCLC cells are consistent with our previous work (4, 5).

Taken together, SCLC cell lines NCI-H69, 209, 378, and 510 express both the myc and bcl-2 proteins. NCI-H345 expresses the bcl-2 protein but not the myc protein. In contrast, NCI-H82 does not express the bcl-2 protein but does express the c-myc protein. Thus, bcl-2 expression is not always associated with myc expression in SCLC cells.

Implications of bcl-2 and myc Expression in SCLC Cells. We have presented data that show relatively high levels of bcl-2 expression in five of six SCLC cell lines. We also showed that the bcl-2 protein expression was not always associated with relatively high levels of myc protein expression in SCLC cells. It should be noted, however, that this study was conducted on SCLC cell lines; therefore, it raises a possibility that some of the SCLC cell lines examined may express higher levels of bcl-2 protein than the original tumor cells. This could occur due to the growth advantage of cells expressing high levels of bcl-2 protein (14). This may also be the case for the expression of myc proteins in these SCLC cell lines. Nevertheless, tumor-derived cell lines often retain the characteristics intrinsic to the original tumor cells. Thus, it is likely that the pattern of bcl-2 and myc expression observed in these SCLC cell lines does reflect the expression pattern of bcl-2 and myc in the original tumor cells. If so, our findings suggest that some proportion, if not the majority, of primary SCLC tumors may express readily detectable levels of bcl-2 and myc proteins. A recent finding of abnormal expression of bcl-2 protein in approximately 20% of non-small cell lung carcinoma tumors, another subset of lung carcinomas, supports this possibility (28). Examination of bcl-2 and myc expression in fresh SCLC tumor specimens will be required to address this question.

Unlike other cancer cells, lung cancer cells in general express low levels of P-glycoprotein, the protein product of MDR-1. Moreover, the P-glycoprotein expression in SCLC cell lines has been found not to correlate with in vitro chemosensitivity of the cells (29). Since high levels of bcl-2 expression promote cell survival in response to a variety of stresses and cytotoxic drugs (8–12), the P-glycoprotein-independent multidrug resistance of SCLC cells may be, at least in part, due to high levels of bcl-2 expression. A recent report that the transfection of the bcl-2 gene into a SCLC cell line resulted in an increase in chemoresistance of the cell (30) supports this idea. If this is the case, the expression of bcl-2 in SCLC cells may become an important indicator in the prognosis or treatment of SCLC. In this regard, it should be mentioned that the expression of bcl-2 protein in non-small cell lung carcinoma patients, especially in the older patients, surprisingly correlates with better prognosis based on five-year survival (28). Thus, further careful studies will be necessary to investigate this issue in SCLC.

In this study, we also found a discordance between the expression of bcl-2 and myc proteins in SCLC cells. NCI-H82 cells express relatively high levels of the c-myc protein, yet it does not express detectable levels of the bcl-2 protein. Since it has been shown that bcl-2 and c-myc cooperate in cell transformation in such a way that bcl-2 suppresses the cell-death-promoting function of c-myc (17, 18), cells like NCI-H82 must have some other mechanisms than bcl-2 to compensate for the cell death promoting function of c-myc, allowing them to survive and grow. Thus, in NCI-H82 cells, some other genes

5 J. Minna, unpublished data.
that are functionally similar to bcl-2 might actually be dysregulated or activated. In fact, such a gene called bcl-x has been identified (31). The bcl-x gene encodes for two proteins by alternative splicing mechanisms. The larger form, called bcl-xL, has a property similar to bcl-2, and it suppresses cell death. Interestingly, the other form, called bcl-xS, dominantly represses the cell-death-sparing function of bcl-2. In addition, yet another gene, called bax, has been identified (32). The bax gene product has been shown to have a similar property to bcl-xS, and bax represses the function of bcl-2 by interacting with it. Thus, it would be of great interest to examine the expression of these newly identified cell death regulators in SCLC in relation to the expression of bcl-2 and myc proteins.

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References

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