COMBINED EVALUATION OF EXPRESSION OF TELOMERASE, SURVIVIN, AND ANTI-APOPTOTIC BCL-2 FAMILY MEMBERS IN RELATION TO LOSS OF DIFFERENTIATION AND APOPTOSIS IN HUMAN HEAD AND NECK CANCERS

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Abstract: Background. Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers, and it accounts for 5% of all adult cancers worldwide. Loss of growth control and a marked resistance to apoptosis are considered major mechanisms driving tumor progression. Little is known about the distribution of inhibitors of apoptosis in HNSCC or how they correlate with other biomarkers of malignancy, such as telomerase, an enzyme that plays a critical role in cellular immortalization. The objective of this study was to assess the protein expression of anti-apoptotic members of Bcl-2 family and survivin and correlate them with telomerase activity.

Methods. We compared anti-apoptotic protein expression in tumor tissue sections of 50 HNSCC patients and 19 histopathologically normal tissues by immunohistochemistry and Western blotting. Apoptotic index was studied by TUNEL assay. Telomerase activity was determined by polymerase chain reaction (PCR)–enzyme-linked immunosorbent assay (ELISA).

Results. Bcl-2, Bcl-XL, and survivin were found to be significantly upregulated in tumor tissues as compared with the normal tissue. Protein expression of Bcl-2 and survivin was significantly associated with the loss of differentiation in tumors and that of Bcl-XL with nodal metastasis. Telomerase activity was found to be upregulated in tumors as compared with the normal tissue ($p < .001$) and was found to be significantly associated with the loss of differentiation in tumors.

Conclusions. Mechanisms that promote both cell proliferation (telomerase activity) and cell survival (expression of inhibitors of apoptosis protein [IAPs]) appear to be activated in HNSCC. This is the first study of its kind to look into the correlation of the apoptotic pathway and proliferation promoting enzyme activity simultaneously in relation to loss of apoptosis and differentiation in HNSCC. Telomerase activity in these tumors was found to be correlated with Bcl-2, Bcl-XL, and survivin overexpression and with reduced apoptosis, thereby suggesting that novel strategies can be developed to control cancer cell growth by co-targeting telomerase and apoptotic pathways.

Keywords: head and neck cancer; apoptosis; Bcl-2; survivin; telomerase

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers, with a global incidence of 500,000 cases per year.1 It accounts for 5% of all adult cancers worldwide. In
India, it accounts for 15.2% of total cancers in males. Despite modern therapeutic strategies like adjuvant and neoadjuvant radiotherapy and chemotherapy in addition to surgical management of the tumor, the overall 5-year survival rate does not exceed 55%. This situation makes the availability of molecular markers highly desirable to guide the use of (neo)adjuvant treatment modalities in the context of clinical studies.

Abnormalities in the control of programmed cell death (apoptosis) play an important role in tumorigenesis. Regulation of apoptosis involves a number of cellular genes including that for B-cell lymphoma 2 (Bcl-2) and related family members. Overexpression of Bcl-2 has been shown to suppress the initiation of apoptosis in response to a number of stimuli, including anticancer drugs. Thus, Bcl-2 is thought to confer a selective growth advantage to tumors when it is overexpressed. Bcl-XL, another member of the anti-apoptotic Bcl-2 family, is a functional and structural homologue of Bcl-2 and provides protection against a wide variety of chemotherapeutic agents. Overexpression of Bcl-XL has been shown to confer drug resistance in some tumor cells and suppress activation of caspases, the cysteine proteases that play a key role in the execution phase of apoptosis.

Several other apoptosis inhibitors related to the baculovirus iap gene have been identified in humans, mice, and Drosophila. Recently, a novel gene encoding a structurally unique inhibitor of apoptosis protein (IAP), designated as Survivin, has been identified. Unlike the anti-apoptotic members of the Bcl-2 family or other IAPs, survivin is undetectable in terminally differentiated adult tissues but becomes notably expressed in common human cancers, including stomach, colorectal, lung, breast, pancreatic, and prostate cancers, and high-grade non-Hodgkin’s lymphomas. Furthermore, a potential therapeutic role is evident from studies demonstrating resistance of survivin-transfected cells to anticancer drug-induced apoptosis and sensitization to chemotherapy by survivin antisense treatment.

In addition to deregulation of apoptosis, increased cell proliferation is also seen in various cancers. Telomerase is a specialized ribonucleoprotein reverse transcriptase that functions in the maintenance of telomeres and is considered necessary for proliferation of cells. Reactivation of telomerase is considered to be one of the key mechanisms involved in cellular immortalization and tumor progression. The present study was undertaken to study the status of survivin, Bcl-2, and Bcl-XL in head and neck squamous cell carcinomas and to investigate the correlation between them, telomerase activity, apoptosis, and clinicopathologic features.

MATERIALS AND METHODS

Sample Collection and Processing. Surgical specimens from 50 HNSCCs and punch biopsy specimens from a normal area contralateral to the site of the lesion of 19 controls were obtained from the Department of Otorhino-laryngology, All India Institute of Medical Sciences (AIIMS), New Delhi, India. The samples were snap frozen and kept at −70°C until further use. The tissue sample was cryocut into 8-μm-thick sections. Concurrent sections were taken for all studies. Histopathology using hematoxylin-eosin staining of these sections was done by a pathologist. Histopathologically confirmed tumor and normal tissues were taken for the study.

Clinicopathologic Characteristics of Patients. Diagnosis of the patients was based on clinical examination and histopathologic analysis of tissue specimens. TNM staging was done based on the Union Internationale Contre le Cancer (UICC) TNM classification of malignant tumors. The tumor samples were graded as well, moderately, and poorly differentiated by a pathologist. The tobacco and/or betel chewing, smoking, alcohol consumption habits and family histories of the patients were recorded.

Immunohistochemical Analysis. Protein expression of anti-apoptotic genes was done by immunohistochemistry using appropriate antibodies, as described earlier. The endogenous peroxidases were first blocked with hydrogen peroxide in phosphate-buffered saline (PBS) (pH 7.4) containing 70% methanol, and nonspecific binding was blocked using 5% bovine serum albumin (BSA). The sections were then incubated with antibodies against Bcl-2, Bcl-XL, and survivin (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:100 in 5% BSA for 48 hours at 4°C. Immunodetection was achieved by an avidin-biotin horseradish peroxidase-based colorimetric method (Vectastain Elite kit from Vector Laboratories, Burlingame CA) with 3,3′-diaminobenzidine (DAB) as a chromogen and H2O2 as the substrate, followed by light counterstaining with hematoxylin and examination under a microscope. Tonsil
FIGURE 1. (A) Immunohistochemical staining for Bcl-XL in normal tissue and well-, moderately, and poorly differentiated HNSCCs (original magnification ×200). Positive (original magnification ×400) and negative controls included are also shown. 3,3'-Diaminobenzidine was used as a chromogen. (B) Immunohistochemical staining for Bcl-2 in normal tissue and well-, moderately, and poorly differentiated HNSCCs (original magnification ×200). Positive (original magnification ×100) and negative controls included are also shown. 3,3'-Diaminobenzidine was used as a chromogen. (C) Immunohistochemical staining for survivin in normal tissue and well-, moderately, and poorly differentiated HNSCCs (original magnification ×200). Positive (original magnification ×300) and negative controls included are also shown. 3,3'-Diaminobenzidine was used as a chromogen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
tissue served as a positive control for Bcl-2 and Bcl-XL, whereas placental tissue was used as a positive control for survivin. The negative controls lacked the primary antibody. The protein expression was determined semiquantitatively. Specimens were considered immunopositive if at least 5% of tumor cells/normal tissue cells displayed distinct immunostaining. Scoring was done by microscopic examination of randomly selected fields containing at least 300 cells. Scoring of immunopositivity was done on the basis of percentage as well as the intensity of staining as follows: 1 AU (arbitrary unit), 5–10%; 2 AU, 11% to 25%; 3 AU, 26% to 50%; and 4 AU, >50%. The intensity of staining was scored as follows: 1 AU, weak; 2 AU, moderate; and 3 AU, intense. The final score was determined by adding up the scores of percentage and intensity of staining.

**Western Blot Analysis.** Normal and tumor tissue were cryocut and lysed in radio immuno precipitation assay (RIPA) lysis buffer containing protease and phosphatase inhibitors. Equal amounts of protein extracts were electrophoresed on 10% to 18% sodium dodecyl sulfate (SDS)–polyacrylamide gels and electrotransferred to nitrocellulose membrane. The membrane was then incubated in 5% BSA for 3 hours followed by overnight incubation with antibodies against rabbit Bcl-2, Bcl-XL, survivin (Santa Cruz Biotechnology), and mouse α-actin (Oncogene Science, Cambridge, MA). α-Actin was used as a loading control. The membrane was then washed twice with Tris-buffered saline (TBS) containing Tween-20 and then with TBS alone for 10 minutes each. Anti-rabbit and anti-mouse alkaline phosphatase conjugated antibodies were then added to the blot and incubated for 2 hours. After washing the membrane, color development was done using 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) substrate from Promega Corporation (Madison, WI). The bands were analyzed and quantitated using a Bio-Rad scanning densitometer. Positive controls used for immunohistochemistry were also included. The protein expression was expressed in terms of relative units (RU). One relative unit is the ratio obtained by taking the density between a positive control and a negative control.22

**TUNEL Assay.** Apoptotic cells were visualized by the terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) technique15 using the Dead End Colorimetric Cell Death Detection kit (Promega Corp.). The sections were incubated with Proteinase K for 20 minutes at room temperature. TdT was used to catalyze the addition of biotin-conjugated dUTP to the 3′-OH ends of DNA fragments. The incorporated biotin was detected by streptavidin conjugated to horse-radish peroxidase. The staining was then done using 3,3′-diaminobenzidine (DAB) as the chromogen and H2O2 as the substrate. The sections were then counterstained with hematoxylin. The apoptotic index was determined by microscopic examination of randomly selected fields containing at least 500 cells. The results are expressed as the percentage of apoptotic cells in 500 cells.

**PCR-ELISA Assay for Telomerase Activity.** Telomerase activity was assayed by using the telomerase polymerase chain reaction (PCR)–enzyme-linked immunosorbent assay (ELISA) kit (Roche Molecular Biochemicals, Mannheim, Germany).16

**FIGURE 2.** (A) TUNEL staining in normal and tumor tissue (original magnification ×200). 3,3′-Diaminobenzidine was used as a chromogen. (B) Western blots for Bcl-2, Bcl-XL, survivin, and α-actin in normal and well-, moderately, and poorly differentiated tissue. Nitroblue tetrazolium was used as a chromogen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
Briefly, the samples were first lysed with a lysis buffer. Telomeric repeat sequences were amplified by PCR using the biotin-labeled P1-TS primer and the P2 primer for generating the telomerase-specific six-nucleotide repeat PCR product. A 5-μL amount of the PCR product was denatured and hybridized to a digoxigenin (DIG) labeled, telomeric repeat-specific detection probe. The resulting product was immobilized via the biotin-labeled primer to a streptavidin-coated microtiter plate. The immobilized PCR product was then detected with an antibody against digoxigenin that is conjugated to peroxidase. Finally, the probe was visualized by adding 3,3′,5,5′-tetramethylbenzidine (TMB) substrate, which is metabolized by peroxidase to a blue-colored product. The absorbance of the samples was then measured at 450 nm (reference filter 690 nm) in an ELISA reader.

### Statistical Analysis
Statistical analysis of the samples was done using SPSS version 10.0 software. The unpaired t test was used to analyze the difference in the parameters between normal and tumor samples. Chi-square statistics were used to test for correlations between the results of immunohistochemical staining, telomerase activity, apoptotic index, and clinicopathologic features.

### RESULTS

#### Immunohistochemical Analysis of Bcl-XL, Bcl-2, and Survivin
Bcl-XL, Bcl-2, and survivin were significantly (p < .001) overexpressed in tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no. of cases</th>
<th>Bcl-2 expression</th>
<th>Bcl-XL expression</th>
<th>Survivin expression</th>
<th>Telomerase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19</td>
<td>26.3 (5)</td>
<td>31.5 (6)</td>
<td>0</td>
<td>36.8 (7)</td>
</tr>
<tr>
<td>HNSCC</td>
<td>50</td>
<td>68 (34)</td>
<td>72 (36)</td>
<td>86 (43)</td>
<td>86 (43)</td>
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<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>17</td>
<td>76.4 (13)</td>
<td>70.5 (12)</td>
<td>82.3 (14)</td>
<td>82.3 (14)</td>
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<tr>
<td>&gt;50</td>
<td>33</td>
<td>63.6 (21)</td>
<td>72.7 (24)</td>
<td>87.8 (29)</td>
<td>87.8 (29)</td>
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</tr>
<tr>
<td>Male</td>
<td>40</td>
<td>65 (26)</td>
<td>72.5 (29)</td>
<td>82.5 (33)</td>
<td>87.5 (35)</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>80 (8)</td>
<td>70 (7)</td>
<td>100 (10)</td>
<td>80 (8)</td>
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<tr>
<td>Histopathologic Grade</td>
<td></td>
<td></td>
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<tr>
<td>Well differentiated</td>
<td>14</td>
<td>50 (7)</td>
<td>64.2 (9)</td>
<td>71.4 (10)</td>
<td>71.4 (10)</td>
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<td>Moderately differentiated</td>
<td>31</td>
<td>74.2 (23)</td>
<td>74.2 (23)</td>
<td>90.3 (28)</td>
<td>90.3 (28)</td>
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<tr>
<td>Poorly differentiated</td>
<td>5</td>
<td>100 (5)</td>
<td>60 (3)</td>
<td>100 (5)</td>
<td>100 (5)</td>
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<td>Tumor stage</td>
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<tr>
<td>T1/T2</td>
<td>7</td>
<td>71.4 (5)</td>
<td>57 (4)</td>
<td>85.7 (6)</td>
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<tr>
<td>T3/T4</td>
<td>43</td>
<td>67.4 (29)</td>
<td>74.4 (32)</td>
<td>86 (37)</td>
<td>86 (37)</td>
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<td>Nodal metastasis</td>
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</tr>
<tr>
<td>N0/N1</td>
<td>38</td>
<td>65.7 (25)</td>
<td>65.7 (25)</td>
<td>84.2 (32)</td>
<td>86.8 (33)</td>
</tr>
<tr>
<td>N2/N3</td>
<td>12</td>
<td>75 (9)</td>
<td>91.6 (11)</td>
<td>91.6 (11)</td>
<td>83.3 (10)</td>
</tr>
<tr>
<td>Site</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td>38</td>
<td>63.1 (24)</td>
<td>68.4 (26)</td>
<td>84.2 (32)</td>
<td>86.8 (33)</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>12</td>
<td>83.3 (10)</td>
<td>83.3 (10)</td>
<td>91.7 (11)</td>
<td>83.3 (10)</td>
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<td>Apoptotic index</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>0.63 ± 0.27</td>
<td>2.54 ± 0.27</td>
<td>3.55 ± 1.02</td>
<td>0.99 ± 0.17</td>
</tr>
</tbody>
</table>

**Table 2.** The immunohistochemical expression of Bcl-2, Bcl-XL, and survivin expression and telomerase activity in controls and tumors.

<table>
<thead>
<tr>
<th>Controls</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2 (AU)</td>
<td>0.63 ± 0.27</td>
</tr>
<tr>
<td>Bcl-XL (AU)</td>
<td>1.05 ± 0.27</td>
</tr>
<tr>
<td>Survivin (AU)</td>
<td>0.00</td>
</tr>
<tr>
<td>Apoptotic index</td>
<td>3.55 ± 1.02</td>
</tr>
<tr>
<td>Telomerase activity (RU)</td>
<td>0.84 ± 0.14</td>
</tr>
</tbody>
</table>

Abbreviations: AU, arbitrary units; RU, relative units.
compared with normal controls (Table 2, Figures 1A and 2B). Bcl-XL immunostaining was detected in 36 (72%) of 50 HNSCCs, although staining intensity varied widely among tissue specimens. A significant association was seen between Bcl-XL overexpression and nodal metastasis ($p = .033$) (Table 1). Bcl-2 immunostaining was detected in 34 (68%) of 50 HNSCCs. Bcl-2 was also significantly ($p < .001$) overexpressed in tumors as compared with the normal controls (Table 2 and Figure 1B). Bcl-2 overexpression significantly ($p = .021$) correlated with the degree of differentiation of the tumor (Table 1 and Figure 2B).

Survivin immunostaining was detected in 43 out of 50 HNSCCs (86%) (Table 1). Survivin was significantly ($p < .001$) overexpressed in tumors, whereas no expression of survivin was seen in normal controls (Table 2). Survivin expression was also significantly associated with the degree of differentiation of the tumors ($p = .021$) (Table 1 and Figure 1C). A highly significant correlation ($p < .001$) was seen between the anti-apoptotic proteins. However, no significant association was seen with tumor size, nodal metastasis, age, sex, smoking index, or alcohol consumption of these patients (Table 1). Western blot analysis corroborated the results of immunohistochemistry (Figure 2B).

**Telomerase Activity.** Forty-three (86%) of 50 HNSCCs were positive for telomerase activity. It was found to be significantly high in tumors as compared with the normal controls ($p < .001$) (Table 1). A positive correlation between telomerase activity and Bcl-2 ($p = .033$), Bcl-XL ($p = .049$), and survivin ($p = .004$) was seen. Telomerase activity was also found to be correlated with the degree of differentiation of the tumors ($p = .01$). No correlation was seen with the other parameters investigated (Table 1).

**Apoptotic Index.** The tumors had a significantly lower apoptotic index as compared with the controls ($p = .001$) (Table 1). Apoptotic index was found to correlate inversely with Bcl-2 ($p = .013$) and survivin ($p = .001$) as well as with telomerase activity ($p = .004$).

**DISCUSSION**

The molecular pathogenesis of HNSCC is still partially understood, and genetic alterations affecting various proto-oncogenes or tumor suppressor genes have frequently been detected. To the best of our knowledge, this is the first study to simultaneously investigate the protein expression and correlation of the anti-apoptotic members of the Bcl-2 family, survivin, and proliferation-promoting telomerase enzyme activity in relation to loss of apoptosis in HNSCC. Immunochemistry has generally been performed on paraffin-embedded archival samples. In this study, frozen-tissue cryosections were used for immunohistochemistry and western blotting, as staining intensity and sensitivity are reported to decrease when tested on paraffin sections as compared with frozen sections. More importantly, telomerase activity assay cannot be performed on paraffin-embedded sections.

Cells harboring multiple genetic alterations are normally eliminated by apoptosis. Diminished apoptosis plays a critical role in tumor initiation, progression, and drug resistance. Several proteins that inhibit apoptosis have been identified, including Bcl-2 family members Bcl-2 and Bcl-XL and the IAPs. Among the recently described IAP family, survivin is characterized by a unique structure and a selective distribution in most human cancers but not in normal tissue.

The results of the present study suggest that decreased apoptosis partially correlated with survivin expression. Specific staining for survivin was detected in 86% cases whereas the normal tissue or the infiltrating lymphocytes did not express survivin as has been previously reported in other studies. Survivin is expressed in a cell cycle regulated manner in the G2-M phase of the cell cycle and associates with microtubules of the mitotic spindle. Disruption of survivin–microtubule interactions results in loss of survivin’s anti-apoptotic function and increased caspase-3 activity during mitosis. The overexpression of survivin in HNSCC may obliterate this apoptotic checkpoint and allow aberrant progression of transformed cells through mitosis. One of the interesting findings of this study was that the expression of survivin in HNSCC was significantly associated with reduced apoptosis, as compared with that in survivin-negative tumors, thereby confirming the anti-apoptotic role of survivin.

The overexpression of the anti-apoptotic members of the Bcl-2 family, Bcl-2 and Bcl-XL, and the inverse correlation of Bcl-2 expression with apoptotic index in tumors suggests that these genes play an important role in overcoming apoptosis in HNSCC. We have earlier reported a similar finding for esophageal cancer.
We found compelling evidence that the presence of survivin in HNSCC was strongly associated with expression of Bcl-2 and with reduced apoptosis. Our results agree with the findings of previous investigations, which showed a similar association between survivin and Bcl-2 expression in neuroblastoma, gastric cancer, colorectal cancer, and high-grade non-Hodgkin’s lymphoma. The survivin gene is encoded at chromosome 17q25, whereas the Bcl-2 gene is located at chromosome 18q21 and may be involved in the tumorigenic t(14;18) translocation. These data imply that other transcriptional factors may contribute to the regulation of both gene products in the progression of cancer. However, regardless of the pathway of the simultaneous coexpression, it appears that survivin and anti-apoptotic members of the Bcl-2 family may mediate non-overlapping, anti-apoptotic mechanisms. Although Bcl-2 and Bcl-XL are integral inner mitochondrial membrane proteins implicated in counteracting cytochrome c release from the mitochondria, IAP molecules, particularly survivin, prevent apoptosis by targeting the terminal effectors caspase-3 and caspase-7. In HNSCC and in many human cancers, inhibition of apoptosis is a general feature, and expression of survivin alone or in conjunction with anti-apoptotic genes like Bcl-2 and Bcl-XL may cause more pronounced anti-apoptotic effects, as reflected in the significantly reduced apoptotic index observed in this study.

Another interesting finding of the significant association between Bcl-2 and survivin, and the histologic grade of tumors, points toward an increase in their expression in poorly differentiated tumors. Our results suggest the differentiation-related increase in survivin and Bcl-2 expression in HNSCC, validating a previous report. An increase in survivin and telomerase activity has also been reported in glioblastomas.

In addition to deregulation of apoptosis, activation of telomerase is also seen in cancer. Reports from our laboratory and others have shown the involvement of telomerase in acquisition of immortality in cancer cells. Our results agree with previous reports of increased telomerase activity in HNSCC, thereby suggesting that telomerase activation plays an important role in the malignant transformation of HNSCC.

Reports indicate that the stable overexpression of the anti-apoptotic Bcl-2 in human cancer cells such as HeLa, which have low Bcl-2 expression, was accompanied by increased levels of telomerase activity, thereby suggesting the regulation of telomerase activity by an apoptotic pathway. In our study, telomerase activity was also found to be correlated with Bcl-2, Bcl-XL, and survivin overexpression and with reduced apoptosis in tumors. This would open new possibilities to develop novel strategies to control cancer cell growth by co-targeting both pathways. Antisense RNA for Bcl-2, Bcl-XL, and survivin have been shown to increase apoptotic cell death in transformed cells. In this context, in addition to chemotheraphy and radiotherapy, targeted antagonists of survivin may be beneficial as apoptosis-based therapy for HNSCC. Currently, we are investigating whether antisense for these proteins can be used as neoadjuvant therapy for the treatment of HNSCC. Telomerase activity inhibition has also been shown to induce apoptosis. Delivering antisense telomerase RNA is an effective antineoplastic gene therapeutic strategy that significantly suppressed the malignant phenotype and enhanced apoptosis of human breast cancer cells. However, a study with a larger cohort is required to firmly establish the utility of looking for expression of Bcl-2, Bcl-XL, survivin, and telomerase activity as molecular markers in HNSCC.

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REFERENCES

10. Tanaka K, Iwamoto S, Gori G, Nohara T, Iwamoto M, Tanigawa N. Expression of survivin and its relationship to...


