ABSTRACT

To determine a cellular factor supporting the survival of gastric cancer cells, a comparative study was performed using two human adenocarcinoma cell lines, SNU-16 and SNU-620. The latter cells were significantly less susceptible to various lethal stimuli including anti-Fas, H$_2$O$_2$, etoposide, and serum withdrawal than the former. These stimuli were found to kill the SNU-16 cells by activating stress-activated protein kinase (SAPK)/c-Jun NH$_2$-terminal kinase (JNK), whereas SAPK/JNK activation was not efficiently induced in the SNU-620 cells. Western blot analysis revealed that Bcl-w, but not the other tested members of the Bcl-2 family, was expressed in the SNU-620 cells to levels higher than that observed in SNU-16 cells. An elevation of the Bcl-w levels in the SNU-16 cells by its stable transfection attenuated both the SAPK/JNK activation and the cell death induced by all of the tested stimuli. These results suggest that the susceptibility of gastric cancer cells to death stimuli is determined, at least in part, by the levels of Bcl-w that suppress the cell death by blocking SAPK/JNK activation. To examine whether Bcl-w was expressed in patients, tumor specimens were obtained from 50 consecutive advanced gastric adenocarcinoma cases. An immunohistochemical analysis showed that Bcl-w was expressed in cancer cells but not in the neighboring normal mucosa of the 23 cases (46%). Interestingly, Bcl-w expression was associated significantly with certain histopathological characteristics of the cancer, notably with the infiltrative morphotypes (P < 0.001). Therefore, Bcl-w appears to be important for gastric cancer cell survival, particularly in infiltrative tumors.

INTRODUCTION

Gastric cancer is one of the most frequent neoplasms with a worldwide distribution (1). The development of a gastric malignancy appears to involve a dysregulation of cell death. A proposed model suggests that during gastric inflammation as a result of a Helicobacter pylori infection or other causes, such a clone of gastric epithelial cells that have achieved a survival advantage against the harmful environment may outgrow and develop into a malignancy (2, 3). This model is consistent with the observation that in many cases, gastric cancer cells respond poorly to anticancer treatments (4, 5). Therefore, to understand the pathogenesis of gastric cancer and to develop new therapeutic strategies, it is essential to determine the cellular components that can support the survival of gastric cancer cells.

The Bcl-2 family of proteins are key regulators of cellular viability (6, 7). Certain members of the family such as Bcl-2, Bcl-X$_L$, and Bcl-w support cell survival, and others such as Bax, Bid, Bak, and Bad promote cell death. Whereas some of these members have been investigated extensively to determine both the molecular mechanisms of their actions and their roles in normal and pathologic conditions, others have received much less attention. Bcl-w is one such member that has been poorly characterized. In particular, there is little information available on the mechanisms whereby Bcl-w suppresses cell death. Given that the ectopic expression of Bcl-w attenuates the hematopoietic cell death induced by interleukin 3 deprivation, γ-irradiation, and dexamethasone (8), Bcl-w may block a lethal signal commonly triggered by diverse forms of stimuli. However, this possibility has not been investigated. Information concerning the physiological roles for Bcl-w is also limited. Whereas Bcl-w expression was detected in various tissues including the brain, testis, heart, and intestines of wild-type mice (8, 9), Bcl-w knockout mice displayed an abnormality only in spermatogenesis (10, 11). This suggests that Bcl-w is dispensable for all of the other developmental processes in normal conditions. However, when Bcl-w-deficient mice were exposed to either γ-irradiation or cytotoxic drugs, their gut epithelial cells underwent apoptosis in a manner more sensitive than the wild-types (12). Therefore, Bcl-w appears to be important under stress conditions at least in the murine intestines. Bcl-w may also play a role in a pathologic condition, particularly cancer. For example, Bcl-w was expressed at relatively high levels in certain tumor cell lines of an epithelial origin, such as colonic, cervical, and breast cancer cells (9).

Bcl-w expression was also observed in a majority of colorectal cancer samples obtained from patients but not in normal mucosal samples (13). Although these observations support a role for Bcl-w in colorectal cancer, Bcl-w expression in other malignant tissues has not been analyzed systematically.

This study was performed to determine the cellular component that can support gastric cancer cell survival. Bcl-w was found to be expressed in human gastric adenocarcinoma cell lines and acts as a cytoprotector by attenuating the ability of various death stimuli to activate the SAPK/JNK, a member of the MAPK family. Bcl-w expression was also detected in nearly half (46%) of patients with an AGC (50 cases) in a manner specific to cancer cells. Interestingly, Bcl-w expression was dramatically segregated with infiltrative, as opposed to demarcated, morphotypes of the tumor (P < 0.001). The overall data suggest that Bcl-w contributes to gastric cancer cell survival, particularly in infiltrative tumors.

MATERIALS AND METHODS

Antibodies. The antibodies against Bcl-2, Bid, Bad, Bax, Bak, and SAPK were purchased from PharMingen/Transduction Laboratories (San Diego, CA). The Bcl-w, ERK, and P38 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-Fas and anti-Bcl-X$_L$ were supplied by Upstate Biotechnology (Lake Placid, NY) and Calbiochem (La Jolla, CA), respectively.

Cell Culture, DNA Transfection, and Treatments. The SNU-16 and SNU-620 cells were cultured in a RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum.
Tina 2.0 software was used to analyze the results. The proteins were separated by 12% SDS-PAGE. A PhosphorImager using incubation time, the reaction was quenched by adding a boiled sample buffer, recombinant c-Jun protein (New England Biolabs, Beverly, MA) for SAPK, the MKK4 mutant were selected using G418 sulfate (1 mg/ml). The cell electroporation. Subsequent treatments with hygromycin (0.5 mg/ml) were received PI (5 μg/ml) followed by flow cytometry analysis to monitor the PI uptake and cell size simultaneously. The cells displaying both a normal size and a low permeability to PI were selected to be viable, as defined previously (15). All of the other populations were understood to be dead.

Determination of Fas Expression. The cells were incubated on ice for 10 min in PBS containing 10% fetal bovine serum and 0.1% sodium azide, followed by a treatment with mouse antihuman Fas IgM (3 mg/ml) for 30 min. The cells were washed, resuspended in a 1:30 dilution of FITC-conjugated goat antimouse IgM (DAKO, Glostrup, Denmark) for 30 min, fixed in PBS containing 2% parafomaldehyde, and analyzed by flow cytometry (16).

Western Blot Analysis. The cell lysates were prepared as described previously (17). Equal amounts of the proteins (100 μg) were separated by 15% SDS-PAGE then electrotransfered to the Immobilon membranes (Millipore, Bedford, MA), which were subsequently blotted using the indicated antibodies and visualized by chemiluminescence (enhanced chemiluminescence: Amer sham Pharmacia, Uppsala, Sweden).

In Vitro Kinase Assay. The lysate proteins (400 μg) were immunoprecipitated using the indicated antibodies and resolved in 20 μl of a buffer defined previously (14). The kinase reactions were initiated by adding 2 μg of the recombinant c-Jun protein (New England Biolabs, Beverly, MA) for SAPK, and PHAS-1 (Stratagene, La Jolla, CA) for ERK and P38 (18, 19). After a set incubation time, the reaction was quenched by adding a boiled sample buffer, and the proteins were separated by 12% SDS-PAGE. A PhosphorImager using Tima 2.0 software was used to analyze the results.

Patient Samples. Stomach samples were obtained from 50 consecutive patients undergoing a radical gastrectomy for AGC from February 2002 to April 2002 at the Korea Cancer Center Hospital. The samples were formalin-fixed, paraffin-embedded, and sliced into serial sections. In each case, representative sections including both the tumor and the normal mucosa were selected for additional analyses.

Pathology. The patients were staged according to the TNM classification (20). The gross types of the tumors were classified according to Bormann (20). The histotypes were determined based on the grade of glandular differentiation and also according to Lauren (21). Alternatively, the patterns of tumor growth and infiltration were analyzed under relatively low microscopic magnifications (×20–100) to divide them into the demarcated, infiltrative, and intermediate types, which are defined in the text.

Immunohistochemistry. The tissue sections were deparaffinized and rehydrated using a procedure described previously (13), and incubated in a citrate buffer [0.01 M, pH 6.0] for 1 min at 121°C. The endogenous peroxidase activity was blocked by covering the sections with 3% H2O2/methanol for 15 min. The sections were then incubated in a 1:50 dilution of goat antihuman Bcl-w IgG at 4°C overnight. After washing with PBS containing 0.05% Tween, the tissue sections were incubated in a 1:50 dilution of biotinylated donkey anti-goat IgG (Santa Cruz Biotechnology) for 30 min. The ABC reagents (Santa Cruz Biotechnology) were used to amplify the immunoreactivity that was detected using 3,3’-diaminobenzidine according to the manufacturer’s instructions. The sections were counterstained with Hematoxylin. The stained sections were examined at least three times to solidify the data. The tumors were regarded as positive if >30% of the tumor cells displayed the immunoreactivity.

Statistic Analysis. A Fisher exact test was performed using the SPSS program for Windows Release 10 to analyze both the significance of cell death, and the correlation between Bcl-w immunoreactivity and the clinicopathologic factors of the patients. The level of significance was determined at P < 0.05.

RESULTS

Differential Susceptibility of SNU-16 and SNU-620 Cells to Death Stimuli. The human gastric adenocarcinoma cell lines, SNU-16 and SNU-620, were exposed to various concentrations of anti-Fas, H2O2, and etoposide to characterize the responses of the cells to death stimuli. Alternatively, the cells grown in 10% serum were washed and given fresh medium containing a lower concentration of serum. The cellular viability was determined by flow cytometry 40 h after treatment. Whereas all of these treatments efficiently killed the SNU-16 cells in a dose-dependent manner, the SNU-620 cells were much less susceptible (Fig. 1A). Therefore, the SNU-16 and SNU-620 cells appeared to be a good model system for a comparative analysis of the mechanism that determines the susceptibility of gastric cancer cells to death stimuli.

Patient Samples. Stomach samples were obtained from 50 consecutive patients undergoing a radical gastrectomy for AGC from February 2002 to April 2002 at the Korea Cancer Center Hospital. The samples were formalin-fixed, paraffin-embedded, and sliced into serial sections. In each case, representative sections including both the tumor and the normal mucosa were selected for additional analyses.

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Statistic Analysis. A Fisher exact test was performed using the SPSS program for Windows Release 10 to analyze both the significance of cell death, and the correlation between Bcl-w immunoreactivity and the clinicopathologic factors of the patients. The level of significance was determined at P < 0.05.
SNU-16 and SNU-620 Cells Express Fas to Similar Levels. The differential susceptibility of SNU-16 and SNU-620 cells to anti-Fas could reflect the different levels of cell surface Fas. However, both cells were almost equally positive for Fas staining (~30%; Fig. 1B). Anti-Fas consistently killed <40% of SNU-16 cells even when the treatment was extended for up to 65 h (Fig. 1C). In contrast, a majority of SNU-16 cells lost their viability in response to H₂O₂, etoposide, and SW. Overall, the data suggests that Fas ligation did not efficiently induce the intracellular death pathways in the SNU-620 cells.

Role of SAPK in Gastric Cancer Cell Death. SAPK has emerged as a key mediator of cell death (14, 22–24). To explore the role of SAPK in gastric cancer cells, the SNU-16 cells were exposed to anti-Fas, H₂O₂, etoposide, and a serum-free medium for up to 24 h when lethality by these treatments was evident in some populations of the cells (Fig. 1C). All of these treatments induced an elevation in SAPK activity, as analyzed by the in vitro kinase assay (Fig. 2A). This was not because of a higher SAPK concentration in the samples. Interestingly, anti-Fas, H₂O₂, and etoposide induced rapid and transient SAPK activation, followed by a second peak. A close examination of the early stages revealed that the first peak was obtained 5 min (anti-Fas), 15 min (etoposide), and 30 min (H₂O₂) after the treatments. The second SAPK activation stage was most evident after 8 h (anti-Fas) and 4–8 h (H₂O₂ and etoposide). The SAPK activation induced by SW was also observed as early as 5 min. However, in this case, the SAPK activity underwent a sustained increase up to 24 h. It was proposed that these SAPK activation patterns, in contrast to a single transient activation, supported cell death (23, 24). To confirm this in gastric cancer cells, a dominant-negative mutant of MKK4, a MAPK kinase that can activate SAPK (25), was stably introduced into the SNU-16 cells. Whereas the expression of the MKK4 mutant did not significantly alter the SAPK expression levels (Fig. 2B), it attenuated the ability of anti-Fas, H₂O₂, and etoposide to induce both SAPK activation peaks (Fig. 2C). The sustained SAPK activation by SW was also reduced in the cells expressing the MKK4 mutant. Importantly, when compared with the control transfectants, these cells were significantly less susceptible to anti-Fas, H₂O₂, etoposide, and SW (Fig. 2D). The data suggest that SAPK acts as a common mediator of gastric cancer cell death induced by the tested stimuli.

In contrast to SAPK, other members of the MAPK family, ERK and P38, did not appear to be activated by the same treatments (Fig. 2A). Consistent with this observation, PD 98059 and SB 203580 (1–50 μM), which specifically inhibit ERK (26) and P38 (27), respectively, did not

![Fig. 2. SAPK is a common mediator of the SNU-16 cell death induced by anti-Fas, H₂O₂, etoposide, and SW. A, the SNU-16 cells were exposed to anti-Fas (100 ng/ml), H₂O₂ (50 μM), etoposide (5 μM), and a serum-depleted medium for the indicated periods. In the second lane (<0.5 h), the cells were treated for 5 min (anti-Fas and SW), 15 min (etoposide), and 30 min (H₂O₂) after the treatments.](downloaded_from_cancerres.aacrjournals.org)
suppress the SNU-16 cell death induced by the test stimuli (data not shown). Therefore, ERK and P38 do not appear to be involved in gastric cancer cell death under these experimental conditions.

**Suppression of SAPK Activation in SNU-620 Cells.** On the basis of the crucial role of SAPK, it was expected that a dysregulation of SAPK confers gastric cancer cells with a resistance to death stimuli. To investigate whether such a mechanism operates in the SNU-620 cells, the cellular levels of SAPK in SNU-16 and SNU-620 cells were compared by Western blot analysis. However, these two cells expressed similar levels of SAPK (Fig. 3A). Nevertheless, anti-Fas, H2O2, etoposide, and SW at the doses that efficiently activated SAPK in the SNU-16 cells failed to do so in the SNU-620 cells (Fig. 3B). This shows that these stimuli did not efficiently kill the SNU-620 cells because of their failure to activate SAPK.

**Differential Expression of Bcl-w in SNU-16 and SNU-620 Cells.** This raised the possibility that SNU-620 cells express a protein that can block SAPK activation. Given the ability of Bcl-2 to suppress the SAPK activation under other experimental conditions (28), the Bcl-2 levels in the SNU-16 and SNU-620 cells were compared using Western blot analysis. However, similar Bcl-2 expression levels were observed in both cells (Fig. 4). This implicates the involvement of other members of the Bcl-2 family. Indeed, Bcl-w was expressed at significantly higher levels in the SNU-620 cells than in the SNU-16 cells. The levels of all of the other tested members including Bcl-XL, Bax, Bid, Bad, and Bak were almost equal in both cases. Therefore, it appears that Bcl-w is responsible for the differential susceptibility of SNU-16 and SNU-620 cells to death stimuli.

**Bcl-w Suppresses the Gastric Cancer Cell Death by Blocking SAPK Activation.** To address this possibility, Bcl-w was stably overexpressed in SNU-16 cells (Fig. 5A). This did not significantly alter the SAPK expression levels. However, Bcl-w overexpression reduced the ability of anti-Fas, H2O2, etoposide, and SW to activate SAPK (Fig. 5B) and kill the cells (Fig. 5C). This suggests that Bcl-w protects gastric cancer cells from the tested stimuli by interfering with their ability to activate SAPK.

**Expression of Bcl-w in AGC Patients.** On the basis of the role of Bcl-w in the gastric cancer cell lines, Bcl-w was additionally examined to determine whether it is expressed in gastric cancer patients. To accomplish this, stomach samples were obtained from 50 consecutive AGC patients and analyzed for Bcl-w expression by immunohistochemistry. In 23 cases (46%), 30–70% of the cancer cells exhibited the immunoreactivity against Bcl-w, which were understood to be Bcl-w-positive (Fig. 6; Table 1). Although the immunoreactivity was focally detected in an additional 7 cases, these were neglected because of a minority of the positive cell population. The staining was cytoplasmic and membranous, as reported previously (8, 9). In contrast, there were no cases where the immunoreactivity was detected in the normal mucosa. Therefore, Bcl-w can be expressed in AGC patients in a manner specific to cancer cells.

**Relationship between Bcl-w Expression and Clinicopathologic Characteristics of Patients.** The variation in Bcl-w expression according to the patients could reflect their age and sex. However, this was not supported by a statistical analysis (data not shown). The possibility that Bcl-w expression was associated with the clinicopathological characteristics of the patients was then investigated. The results are summarized in Table 1. The table shows that Bcl-w expression did not correlate with either the TNM stages or the differentiation grades of the tumors. However, Bcl-w expression was associated significantly with Lauren’s tumor classification. Because 76% of the diffuse/mixed types tested positive for the staining, the immunoreactivity was detected in only 30% of the intestinal types (P = 0.002). When stratified for the Borrmann’s gross types, the Bcl-w expression was significantly segregated with the infiltrative tumors (type III and IV, 65%) as opposed to the demarcated tumors (type I and II, 16%; P = 0.001).

During the course of the pathologic analysis, the frequency of Bcl-w expression appeared to vary according to the patterns of tumor cell growth/infiltration, as analyzed by optical microscopy under relatively low magnifications (×20–100). To systemically investigate this possibility, the tumors were classified into demarcated, infiltrative, and intermediate types. The demarcated type was defined as the one showing expansile growth of the tumor mass with a sharp periphery (Fig. 7A). Such a demarcated tumor mass was not observed in the cases where the muscle and interstitial tissues were infiltrated by the individual tumor glands (Fig. 7B) and cells (Fig. 7C). This was defined as the infiltrative type. In the other cases, although tumor

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**Fig. 3.** Suppression of SAPK activation in the SNU-620 cells. A, the SAPK levels in the SNU-16 and SNU-620 cells were compared by Western blotting. B, the SNU-16 and SNU-620 cells were exposed to 100 ng/ml anti-Fas for 5 min and 8 h, 50 μM H2O2 for 30 min and 8 h, 5 μM etoposide for 15 min and 8 h, or a serum-depleted medium for 5 min and 24 h, and their SAPK activities were compared.
masses were relatively well demarcated, their peripheries were not sharp but infiltrating (fuzzy margin). This mixed pattern that appeared more likely to be the demarcated type than the infiltrative was defined as the intermediate type (Fig. 7D). There were 10 demarcated, 18 intermediate, and 22 infiltrative types in the samples analyzed in this study. Bcl-w expression was detected in only 1 and 4 cases of the demarcated (10%) and intermediate types (22%), respectively. In contrast, 18 cases of the infiltrative type tested positive for Bcl-w staining (82%). Statistical analyses confirmed the strong association of Bcl-w expression with the infiltrative type ($P < 0.001$).

**DISCUSSION**

This study showed that the cellular levels of Bcl-w are important determinants for the susceptibility of gastric cancer cells to various death stimuli. This was illustrated by the use of SNU-16 and SNU-620...
cells (29, 30). These gastric adenocarcinoma cell lines were established from the patients of same sex (H20038), ethnic background (Mongoloid), and pathological type (poorly differentiated). Moreover, both cells grow in suspension, although some other gastric cancer cell lines are adhesive. Despite these common features, SNU-620 cells were significantly less susceptible to anti-Fas, H2O2, etoposide, and SW than the SNU-16 cells. This difference appeared to be attributable to Bcl-w because the levels of Bcl-w in the SNU-620 cells were significantly higher than in the SNU-16 cells. The possibility was indeed supported by the observation that Bcl-w overexpression in the SNU-16 cells reduces their susceptibility to all of the tested stimuli. Therefore, Bcl-w appears to protect gastric cancer cells from such types of lethal stimuli, which include ligands for the cellular receptors, cytotoxic drugs, and environmental stresses. This is also supported by previous reports showing that Bcl-w rescued the hematopoietic and gut epithelial cells from dexamethasone, 5-fluorouracil, interleukin 3 deprivation, and γ-irradiation (8, 12). These features of Bcl-w appear to confer the Bcl-w-expressing cells with both an advantage for their expansion against physiological death stimuli and a resistance to anticancer treatments. However, it should be noted that Bcl-w failed to antagonize the lethal action of anti-Fas in the hematopoietic cells (8), which contrasts with the results obtained in this study using gastric cancer cells. Such differences may reflect the nature of the cell types used in the respective studies. Similarly, Bcl-2 suppressed Fas-mediated lethal signaling only in certain cell types (31).

The data suggest that Bcl-w suppresses cell death by blocking SAPK activation. This was initially suggested by the finding that SAPK acts as a common mediator of SNU-16 cell death induced by anti-Fas, H2O2, etoposide, and SW. Interestingly, the ability of these stimuli to activate SAPK was suppressed in the SNU-620 cells, which constitutively expressed Bcl-w at relatively high levels. Similarly, anisomycin (10 μM) efficiently activated SAPK only in SNU-16 but not in SNU-620 cells (data not shown). Moreover, Bcl-w overexpression in the SNU-16 cells attenuated the SAPK activation induced by all of the tested stimuli. To best of our knowledge, this is the first report to demonstrate a mechanism whereby Bcl-w suppresses cell death.

### Table 1 Relationship between Bcl-w expression and clinicopathologic factors

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Bcl-w-positive cases/ no. of total cases (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>2/6 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>II</td>
<td>4/11 (36)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>9/21 (43)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>8/12 (67)</td>
<td></td>
</tr>
<tr>
<td>Grade of differentiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated (including papillary)</td>
<td>7/12 (58)</td>
<td>NS</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>1/10 (10)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>15/28 (54)</td>
<td></td>
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<tr>
<td>Histologic type (Lauren)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>10/33 (30)</td>
<td>0.002</td>
</tr>
<tr>
<td>Diffuse/mixed</td>
<td>13/17 (76)</td>
<td></td>
</tr>
<tr>
<td>Gross type (Bormann)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demarcated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (polypoid)</td>
<td>0/3 (0)</td>
<td>0.006</td>
</tr>
<tr>
<td>II (ulcerofungating)</td>
<td>3/16 (19)</td>
<td></td>
</tr>
<tr>
<td>III (ulceroinfiltrative)</td>
<td>15/24 (63)</td>
<td></td>
</tr>
<tr>
<td>IV (diffuse)</td>
<td>5/7 (71)</td>
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<tr>
<td>Demarcated (3/19, 16%) vs. infiltrative (20/31, 65%)</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Microscopic growth pattern</td>
<td></td>
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<tr>
<td>Demarcated</td>
<td>1/10 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intermediate</td>
<td>4/18 (22)</td>
<td></td>
</tr>
<tr>
<td>Infiltrative</td>
<td>18/22 (82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Demarcated + intermediate (5/28, 18%) vs. infiltrative (18/22, 82%)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*NS, not significant.

**Fig. 7.** Classification of AGC based on the microscopic patterns of tumor cell growth/infiltration. The tissue sections were stained with H&E and were observed under relatively low microscopic magnifications (×20–100). A, demarcated type: the carcinoma shows an expansile growth pattern with a sharply defined periphery and compresses the neighboring normal tissue (×20). B and C, infiltrative type: individual carcinoma glands (B) and cells (C) infiltrate diffusely into the muscularis propria (×100). D, intermediate type: whereas the tumor mass is relatively well circumscribed, its border is not sharp but infiltrating (×40).
death. It was reported previously that Bcl-w could form complexes with Bax and Bak in rat testis cells (32). The ability of Bcl-w to attenuate the release of cytochrome c from the mitochondria was also proposed in rat neuronal cells (33). Whereas these features of Bcl-w potentially underlie additional mechanisms for the cytoprotective function of Bcl-w, this possibility has not been demonstrated directly in the respective model systems. Such a demonstration appears to be important, because cytochrome c release and the subsequent activation of the caspases are not always responsible for cell death (34, 35), although these events are necessarily for inducing certain morphological features of apoptosis (35).

It has been proposed that Bcl-w is intracellularly localized to the mitochondria and nuclear envelopes, the same sites where Bcl-2 resides (8, 9). The ability of Bcl-w to interact with the proapoptotic Bcl-2 family members and to attenuate cytochrome c release is also shared by Bcl-2 (6, 7). The functional analogy between Bcl-w and Bcl-2 was additionally supported by the results in this study showing that Bcl-w, like Bcl-2 (28), inhibits SAPK activation. Moreover, although both Bcl-w and Bcl-2 act against numerous lethal stimuli, their ability to suppress Fas-mediated cell death appears to depend on the cell types, as stated above. Overall, Bcl-w appears to function in a manner similar to Bcl-2.

An important finding of this study is that Bcl-w was expressed not only in the tested gastric cancer cell lines but also in the cancer cells from AGC patients. This was true in almost half (46%) of the tested cases. Moreover, Bcl-w expression was specific to cancer cells and was not detected in the neighboring normal cells. These observations support the notion that Bcl-w can be deregulated in gastric cancer cells to promote their survival. A similar role for Bcl-w was also proposed in colorectal cancers (13). Although Bcl-w was reportedly expressed in certain tumor cell lines originating from other epithelial tissues such as the cervix and breast (9), we failed to observe a Bcl-w-positive case by analyzing the tumor specimens obtained from patients with cervical (10 cases) and breast cancer (15 cases). Other investigators also reported a lack of evidence showing that Bcl-w is expressed in these cancer patients (13). Therefore, within the limits of currently available information, Bcl-w appears to be important in cancer, particularly those in the gastrointestinal tract.

Interestingly, our results suggest that Bcl-w is expressed preferentially in particular histopathological types of gastric cancer. Although Bcl-w expression was not related significantly to the grades of tumor promotion and differentiation, Bcl-w was more frequently expressed in the diffuse/mixed type (76%) than the intestinal type (30%; P = 0.002). Given that the diffuse type of Lauren classification displays an infiltrative morphology in most cases (36), it appears likely that Bcl-w expression was also segregated with the infiltrative cases. This possibility was indeed confirmed when Bcl-w expression was stratified for the Borrmann types. Of the infiltrative cases (type III and IV), 65% tested positive for the Bcl-w-staining. Moreover, the immunoreactivity was detected in only 16% of the demarcated cases (type I and II; P = 0.001). When the tumors were classified according to their microscopic growth/infiltration patterns defined in this study, a more dramatic association of Bcl-w expression with the infiltrative morphotypes (82%) as opposed to the demarcated/intermediate types (18%) was observed (P < 0.001). Therefore, Bcl-w appears to be important for the survival of gastric cancer cells, particularly in patients with the infiltrative morphotypes. This may reflect the possible relationship between Bcl-w expression and the invasiveness of the gastric cancer cells.

The evidence suggests that Bcl-w may not be the sole cytoprotector expressed in gastric cancer cells. For example, it was reported that Bcl-2 was expressed in the gastric cancer cells obtained from patients (37–40). These observations raise a question as to why gastric cancer cells express both Bcl-w and Bcl-2, which function in a very similar manner. In this regard, several research groups, except one (40), consistently proposed that Bcl-2 was expressed more frequently in the intestinal type than in the diffuse type of gastric tumor (37–39). This contrasts with the Bcl-w expression observed in this study. Therefore, Bcl-w and Bcl-2 may be preferentially expressed in different histopathological types of gastric cancer. This possibility is currently under investigation.

In conclusion, the expression and function of Bcl-w in gastric cancer was analyzed at both the cellular and pathologic levels. The results suggest that Bcl-w is expressed in a majority of infiltrative gastric adenocarcinomas, and protects the cancer cells from diverse lethal stimuli by blocking their ability to activate SAPK. These findings significantly advance our understanding not only of Bcl-w but also on the pathogenesis of gastric cancer.

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Bcl-w Is Expressed in a Majority of Infiltrative Gastric Adenocarcinomas and Suppresses the Cancer Cell Death by Blocking Stress-activated Protein Kinase/c-Jun NH₂-terminal Kinase Activation
