Increased Expression and Secretion of Interleukin-6 in Patients with Barrett’s Esophagus

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ABSTRACT

Purpose: Barrett’s esophagus (BE) is a common premalignant lesion of the distal part of the esophagus that arises as a consequence of chronic duodenogastroesophageal reflex. Interleukin (IL)-6 is a pleiotropic cytokine that regulates immune defense mechanisms and hematopoiesis. In addition, IL-6 may also be involved in malignant transformation and tumor progression. IL-6 has been shown to inhibit apoptosis. The major aim of this study was to evaluate expression of IL-6 in BE at the protein and mRNA levels. In addition, we tested whether proteins that are associated with IL-6 signaling, phosphorylated signal transducer and activator of transcription 3 and two antiapoptotic proteins, Bel-2, and Mcl-1, are expressed in the same tissues.

Experimental Design: Biopsies of duodenum, BE, and squamous epithelium were evaluated by using a human cytokine protein array, ELISA, real-time PCR, and immunohistochemistry.

Results: Increased IL-6 levels were found to be secreted from BE tissue compared with duodenum or squamous epithelium from sites adjacent or 5 cm away from the BE lesion. IL-6 mRNA was also elevated in BE compared with duodenum or squamous epithelium in five of seven patients.

Immunohistochemical studies confirmed IL-6 expression in intestinal glandular epithelium in BE tissue. Activated signal transducer and activator of transcription 3, Mcl-1, and Bcl-xL are present at higher levels in BE glands, with lower levels being found in duodenum or squamous epithelium.

Conclusions: These data, taken together, suggest that elevated IL-6 levels in BE may contribute to the development of apoptosis resistance, thereby placing this epithelium at higher risk of developing malignancy.

INTRODUCTION

Barrett’s esophagus (BE) is a common premalignant lesion of the distal part of the esophagus that arises as a consequence of chronic gastroesophageal reflux. BE is estimated to be present in approximately 6–12% of patients undergoing endoscopy for symptomatic gastroesophageal reflux disease (1). Multiple studies have shown that BE is associated with increased risk of esophageal adenocarcinoma (ADCA). The incidence of esophageal ADCA is rising rapidly for unknown reasons in North America and Western Europe (2). This cancer has a poor prognosis, with a median survival of less than 1 year.

Histologically, BE is characterized as a condition where squamous epithelial cells are replaced by metaplastic intestinal-like columnar epithelium containing goblet cells (intestinal metaplasia). However, the mechanism of development of BE is not known. BE appears to result from chronic irritation of esophageal mucosa by gastric acids, proteases, and bile acids (3). Fitzgerald et al. (4) reported recently that Th1 cytokines are increased in esophagitis compared with Th1 cytokine levels in BE and noninflamed squamous tissues. In contrast, BE was characterized by a Th2 cytokine profile [interleukin (IL)-4 and IL-10] and little to no evidence of inflammation (4). However, no studies have been done to evaluate IL-6 expression, a key cytokine associated with a Th2 response. Elevation of IL-6 may lead to the activation of the signal transducer and activator of transcription (STAT) 3 pathway and subsequent increased expression of antiapoptotic genes with resulting apoptosis resistance.

In this study, we evaluated the expression of IL-6 mRNA and protein levels and the secretion of IL-6 from biopsies obtained from duodenum, BE, and squamous epithelium. In addition, we evaluated expression of soluble IL-6 receptor (sIL-6R) and the activation of STAT3 in these tissues as well as the expression of antiapoptotic proteins Bcl-xL and Mcl-1. The data indicate a prominent role for IL-6 in the development of BE and apoptosis resistance.

MATERIALS AND METHODS

Patients. Fifteen patients with known BE were included in the present study (Table 1). All patients gave written informed consent with the approval of the University of Arizona Human Subjects Committee. Endoscopic biopsies of duodenum,
BE, and squamous mucosa were taken from patients undergoing regular surveillance procedures. Adjacent biopsies were stained with H&E and Alcian blue (pH 2.5) for histological evaluation and assessment of intestinal metaplasia, degree of dysplasia, and inflammation. BE was defined as the presence of intestinal-like metaplastic epithelium containing goblet cells (intestinal metaplasia) from the sites above the gastro-esophageal junction. Biopsies for mRNA analysis were taken from seven patients and immediately stored in RNAlater solution (Ambion Inc., Austin, TX). In all patients, the length of BE was measured as the difference between proximal and distal extent (gastro-esophageal junction) of the lesion observed with the video-image endoscope. The experimental design of the study is summarized in Table 1.

### Table 1: Patient characteristics, degree of BE inflammation, and summary of experiments performed with tissue biopsies

<table>
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<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Length (cm)</th>
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<th>Experiments</th>
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<td>6</td>
<td>IM/HGD</td>
<td>IHC, ELISA, RT-PCR, HPCA, INFLAM</td>
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<td>56</td>
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<td>IM</td>
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</table>

*BE, Barrett’s esophagus; IHC, immunohistochemistry; RT-PCR, reverse transcription real-time PCR; HPCA, Human Protein Cytokine Array II; INFLAM, inflammation; ND, not done; IM, intestinal metaplasia; HGD, high-grade dysplasia; LGD, low-grade dysplasia; ID, indefinite for dysplasia; 0, no inflammation; 1, mild inflammation; 2, moderate inflammation.

BE esophagus (4).

### Microscopic Evaluation of Inflammation.

The degree of inflammation was determined in formalin-fixed sections stained with H&E and Alcian blue using the updated Sydney system, which takes into account the quantity of lymphocytic, neutrophilic, or eosinophilic infiltrates, as described previously in BE esophagus by Fitzgerald et al. [0, none; 1, mild; 2, moderate; 3, marked (Ref. 4)].

### Screening of Conditioned Media for Cytokine Secretion.

In a preliminary study, the secretion of various cytokines into conditioned media from duodenum, BE, and normal squamous esophageal epithelium was evaluated in four BE patients using Human Cytokine Protein Array II obtained from RayBiotech, Inc. (Norcross, GA) according to the manufacturer’s protocol. This assay can simultaneously detect 43 different cytokines with high specificity. Biopsies were immediately washed in media consisting of Eagle’s MEM α-modification (MEM; Sigma Chemical Co., St. Louis, MO) supplemented with 0.1 mg/ml kanamycin, 2 mM L-glutamine, and DMSO (0.5 μl/ml) and weighed. Conditioned media were obtained after the incubation of these biopsies for 6 h in 600 μl of MEM at room temperature. The volume of media used for the assay was normalized to reflect the biopsy weight. This is done to adjust for the higher output of larger samples due to size alone. For example, for 10 mg of tissue, 500 μl of media were used in the assay; whereas for 20 mg of tissue, only 250 μl of media were used. After the 6-h incubation, the volume of media was adjusted to 1 ml with fresh media so that the final volume used for cytokine determination in the assay was 1 ml. To determine the relative concentrations of cytokines in the media, the densities of individual spots were measured using the ImagePro software (Media Cybernetics, Silver Spring, MD) for image capturing and analysis. The results were expressed as relative densities compared with positive controls included in each membrane.

The relative increase in IL-6 densities in BE tissue was compared with the relative densities of control tissues (duodenum or squamous epithelium) from the same patient, which were set as 1. The data were analyzed using Student’s t test. In addition, all specimens were fixed in formalin, embedded in paraffin, and evaluated histologically to confirm the histopathological specificity and integrity of the tissue after the incubation.

### Analysis of Conditioned Media for IL-6 Secretion.

Initial screening for differential secretion of cytokines in three different tissues from BE patients revealed consistent increase in the expression of IL-6 in BE biopsies compared with squamous epithelium or duodenum. Thus, ELISAs were used to determine the concentrations of IL-6 and sIL-6R in conditioned media after incubation of the biopsies in media for 3 h. Quantikine HS ELISA kits for the detection of IL-6 and sIL-6R were obtained from R&D Systems, Inc. (Minneapolis, MN), and the assay was performed in duplicate according to the manufacturer’s instructions. Biopsies of duodenum, BE, and two samples of normal squamous mucosa (1 and 5 cm away from the BE lesion) were obtained from 10 patients using a video-image endoscope. In addition, a biopsy was taken from one patient from a large nodule within the BE lesion. This was diagnosed by the study pathologist (A. P.) as esophageal ADCA. All biopsies were
weighed; washed in MEM supplemented with 10% (v/v) heat-inactivated FCS (Omega Scientific, Inc., Tarzana, CA), 2 mM l-glutamine, 5 mM HEPES, 1 mM nonessential amino acids (Sigma Chemical Co.), 100 units/ml penicillin, and 100 μg/ml streptomycin; and then incubated for 3 h in 700 μl of this media at 37°C and 5% CO₂ (5). After incubation, the tissues were fixed in 10% buffered formalin, and aliquots of conditioned media were stored at −80°C until use. The measurements were done in duplicate, and the results were expressed as pg/mg tissue, as described previously by Fitzgerald et al. (4).

**Immunohistochemical Analysis.** For the evaluation of IL-6, STAT3, phosphorylated STAT3, Mcl-1, and Bcl-xL expression, a standard immunostaining assay with a biotin-linked peroxidase detection method was used, as described previously (6). Briefly, 4-μm paraffin-embedded sections were deparaffinized and rehydrated by standard methods. Slides were then placed in 15% hydrogen peroxide for 30 min to block endogenous peroxidase. After antigen retrieval using a saponin or microwave protocol, the slides were incubated in serum for 30 min and immunostained with antibodies against IL-6 (1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), Bcl-xL (1:50; Santa Cruz Biotechnology, Inc.), Mcl-1 (1:50; NeoMarkers, Fremont, CA), STAT3 (1:100; Cell Signaling Technology Inc., Beverly, MA), or phosphorylated STAT3 (1:50; Tyr705; Cell Signaling Technology Inc.) for 1 hr at room temperature. After three rinses with PBS, the secondary antibodies, biotinylated rabbit antimouse or goat antirabbit IgG antibodies (1:400; Dako Corp., Carpinteria, CA) were applied for 30 min. Slides were again rinsed with PBS, andVectastain ABC reagent (Elite PK-6100, Standard; Vector Laboratories, Burlingame, CA) were added according to kit instructions and incubated for 30 min. After three final PBS rinses, slides were immersed in 3,3′-diaminobenzidine (0.25 mg/ml), activated with hydrogen peroxide for 5 min, rinsed, and lightly counterstained with hematoxylin. Sections were then dehydrated with xylene and coverslipped using mounting media Cytoseal XYL (Richard-Allen Scientific, Kalamazoo, MI). A simple grading system (on a scale of 0–3) was used to grade the level of expression of individual proteins, as has been described previously (6).

**Real-Time Reverse Transcription-PCR.** Biopsies from seven patients were immediately transferred to 2 ml of RNA-later solution, and total RNA was isolated using a RNeasy Qiagen Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Real-time reverse transcription-PCR was used to quantify relative IL-6 mRNA levels, as described previously (7). Briefly, after reverse transcription of 1 μg of total RNA, real-time PCR amplification was performed using human IL-6 TaqMan Predeveloped Assay Reagents (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Samples were subjected to 40 cycles of amplification at 95°C for 15 s followed by 1 min at 60°C using a GeneAmp 5700 Sequence Detection System (Applied Biosystems). The PCR reactions for each sample were done in duplicate. All mRNA levels were calculated on the basis of total RNA concentration according to Bustin (8), who has demonstrated problems in using housekeeping genes to normalize mRNA levels. Thus, separate standard curves for IL-6 were generated from serial dilutions of control total RNA (from 1 to 500 ng per reverse transcription reaction).

**Statistical Evaluation.** Data for secreted IL-6 are expressed as mean ± SE. ANOVA was used to compare values between tissues. Because the IL-6 mRNA measurements were not normally distributed, the Wilcoxon signed rank test was used to compare the difference in mRNA between BE and duodenum, BE and adjacent squamous epithelium, and BE and distant squamous epithelium. For this multiple comparison, the P was adjusted by Bonferroni adjustment.

**RESULTS**

**Secretion of IL-6.** In the preliminary screening protocol, duodenum, BE, and squamous epithelium tissues from four patient were evaluated for the secretion of cytokines in Human Cytokine Protein Array II, an assay that can detect 43 different cytokines (Fig. 1). The relative densities of individual signals were measured and compared with the relative densities of control tissues, duodenum or squamous epithelium, from the same patient. We found significantly increased IL-6 levels in conditioned media obtained after 6 h of incubation from BE tissues compared with duodenum or squamous epithelium (P < 0.05). In summary, the IL-6 relative densities in BE were 1.4 ± 0.2 and 1.6 ± 0.3, compared with duodenum and squamous epithelium, respectively. The relative densities of duodenum and squamous epithelium were set equal to 1.

These results were confirmed by ELISA. IL-6 levels were measured in conditioned media from biopsies obtained from duodenum, BE, and squamous epithelium from 10 different BE patients. Our results indicate that BE tissue from all 10 patients secreted elevated amounts of IL-6 (15.3 ± 13.4 pg/mg) into the media compared with squamous epithelium or duodenum. Lower mean levels of IL-6 were detected in conditioned media from duodenum (1.2 ± 1.7 pg/mg) and squamous epithelium adjacent to BE (1.1 ± 1.2 pg/mg) or from squamous epithelium 5 cm away from BE (0.8 ± 0.9 pg/mg; Fig. 2A). The difference was statistically significant (P < 0.05) when BE was compared with duodenum or squamous epithelium adjacent to BE or distant from BE. Despite a wide range of values for BE from patient to patient, it was always greater than that in the control tissues (Fig. 2B). Two patients (patients 3 and 9) also exhibited an increase in IL-6 secreted from squamous epithelium adjacent to BE, indicating a possible field defect (9).

**Secretion of sIL-6R.** In addition, secretion of sIL-6R was evaluated in the conditioned media from these tissues (Fig. 3). The highest mean level of sIL-6R was found in media obtained from the duodenum (37.7 ± 8.5 pg/mg), whereas in media from BE, squamous epithelium adjacent to BE lesion, and squamous epithelium 5 cm away from BE, the mean levels were 8.3 ± 4.4, 7.7 ± 6.7, and 2.8 ± 1.7 pg/mg, respectively (Fig. 3A). The levels of secreted sIL-6R did not vary as widely among the individual patients (Fig. 3B) as the levels of IL-6 (Fig. 2B).

**Inflammation.** The biopsies adjacent to the studied biopsies from 10 patients were scored for inflammation as described previously (4). Based on the degree of lymphocytic, neutrophilic, and eosinophilic infiltration, we quantitatively estimated the inflammation as none (0), mild (1), moderate (2), or marked (3). As reported previously by Fitzgerald et al. (4, 10), we found absent to mild inflammation in BE biopsies (Table 1). Interestingly, the inflammation score was highest in duodenum, whereas in squa-
mous epithelium tissues, no inflammation or mild inflammation was detected.

**IL-6 mRNA Levels.** Additional biopsies from seven patients were obtained for mRNA studies. The tissues evaluated included duodenum, BE, and squamous epithelium adjacent to and 5 cm away from BE lesions. IL-6 mRNA levels were elevated in the BE lesion of five of seven patients compared with all other tissues. The mean levels of IL-6 mRNA were elevated in BE (290.2 ± 541.6) and squamous epithelium adjacent to BE (251.1 ± 623.4) compared with the low levels in duodenum (16.3 ± 13.9) and squamous epithelium 5 cm away.

**Fig. 1** Images of Human Cytokine Protein Array II membranes that were assayed with conditioned media obtained after incubation with duodenum (A), Barrett’s esophagus (B), and squamous epithelium (C) for 6 h. The interleukin-6 signal is indicated by a rectangle in each image.

**Fig. 2** Interleukin (IL)-6 secretion from different tissues. A, the mean ± SE of IL-6 levels secreted in 3 h by different tissues obtained by ELISA assay from all 10 patients; the asterisk indicates a statistically significant difference in the mean value compared with Barrett’s esophagus (BE). B, IL-6 levels found in the conditioned media after a 3-h incubation of different tissues in 10 individual patients. In several tissues, the SE was too small; hence, the error bars are not seen. □, duodenum; □, BE; ■, squamous epithelium adjacent to the BE lesion; ■, squamous epithelium away from the BE lesion.

**Fig. 3** Secretion of soluble interleukin-6 receptor (sIL-6R) from different tissues. A, the mean ± SE of sIL-6R concentrations in conditioned media obtained from different tissues after a 3-h incubation by ELISA. B, sIL-6R levels found in the conditioned media after a 3-h incubation in 10 individual patients. □, duodenum; □, Barrett’s esophagus (BE); ■, squamous epithelium adjacent to the BE lesion; ■, squamous epithelium away from the BE lesion.
from the BE lesion (24.3 ± 19.0; Fig. 4A). However, the variability among the individual patients was high (Fig. 4B). The level of IL-6 mRNA in BE in one patient (patient 5) was especially high (Fig. 4B). Interestingly, this patient developed esophageal cancer 2 months later. In the squamous epithelium adjacent to BE, we found low levels of IL-6 mRNA in the majority of patients; however, in one patient (patient 9), markedly elevated levels of IL-6 mRNA were detected. Due to highly elevated levels of mRNA in one BE biopsy and one squamous epithelium adjacent to the BE lesion, the difference between IL-6 mRNA levels in BE and adjacent squamous epithelium did not reach statistical significance (P > 0.05). The difference was statistically significant when BE was compared with duodenum or squamous epithelium 5 cm away from the BE lesion (P < 0.05).

IL-6 mRNA and Protein in Esophageal ADCA. The histological diagnosis of the nodule within the BE lesion from patient 7 was esophageal ADCA. Interestingly, the cytokine analysis of conditioned media obtained from this cancerous tissue indicated that the IL-6 concentration was increased compared with BE, duodenum, or squamous epithelium from the same patient (Fig. 5A). The IL-6 mRNA levels were also markedly elevated in the ADCA compared with the IL-6 mRNA concentrations in BE, duodenum, or squamous epithelium from the same patient (Fig. 5B).

Immunohistochemical Studies. To confirm the expression of IL-6 and determine the cell types that secrete IL-6 from BE tissue, immunohistochemical staining with a polyclonal IL-6 antibody was used. We found that IL-6 is expressed in epithelial cells of BE tissue (Fig. 6). In contrast, duodenum and squamous epithelium adjacent to BE showed milder IL-6 staining. Faint IL-6 staining was also observed in squamous epithelium 5 cm away from the BE lesion (Fig. 6).

Because IL-6 induces phosphorylation of STAT3, and this activation of STAT3 is associated with increased levels of several antiapoptotic genes, we evaluated the expression of phosphorylated STAT3, Bcl-xL, and Mcl-1 in the duodenum, BE, and squamous epithelium. Immunohistochemical analysis revealed intense staining of phosphorylated STAT3 in the epithelial cells in BE biopsies, with marked nuclear staining indicating the nuclear translocation of activated STAT3 (Fig. 6). Nuclear staining of phosphorylated STAT3 was observed in the basal cell layers only of the adjacent and distant squamous epithelium, whereas no staining was detected in the upper layer of epithelial cells (Fig. 6). In duodenum, the positive nuclear staining of activated STAT3 was scattered in several epithelial cells.

Both antiapoptotic proteins evaluated, Mcl-1 and Bcl-xL, were expressed in epithelial cells of BE (Fig. 6). In BE lesions, the expression of Mcl-1 was markedly more intense than that in the duodenum or squamous epithelium and showed distinct nuclear localization (Figs. 6 and 7). Mcl-1 was also expressed in the basal layers of squamous epithelium, but no staining was observed in the upper layers of squamous epithelium (Fig. 6). Bcl-xL was expressed focally in the dysplastic regions of BE, whereas lower Bcl-xL staining was detected in squamous epi-
thelium (Fig. 6). In duodenal biopsies, Bcl-xL was moderately expressed in the majority of patients (Fig. 7).

**DISCUSSION**

IL-6 is a pleiotropic cytokine that has been shown to regulate immune defense mechanisms and hematopoiesis (11). In addition, IL-6 appears to be involved in malignant transformation and tumor progression (11). This cytokine is produced not only by immune cells but also by other cell types, including epithelial cells and osteoblasts (12). Expression of IL-6 by cancer cells suggests that IL-6 may act as an autocrine growth factor to promote tumor progression by inducing the expression of antiapoptotic and angiogenic factors (13–17). Its overexpression has been implicated in the pathogenesis and prognosis of different tumors including multiple myeloma and cancers of the ovary, prostate, breast, kidney, and lung (18–24). In addition, several studies demonstrated that IL-6 and the IL-6 receptor are expressed in intestinal epithelial cells and that these proteins are also increased in colorectal cancers (25–27). IL-6 was also shown to stimulate clonogenic growth of human colon carcinoma cells (28). Wang *et al.* (29) have shown increased mRNA levels of IL-6 in paraffin sections using *in situ* hybridization and increased serum IL-6 levels using ELISA in patients with esophageal squamous cell carcinoma. Constitutive STAT3 activation was demonstrated to have a role in oncogenesis, and activated STAT proteins are found to be present in various human cancers (17, 30–32).

Fitzgerald *et al.* (4) recently reported that despite a lack of inflammation, BE tissues express elevated amounts of Th2 cytokines (IL-4 and IL-10). In contrast, Th1 cytokines are expressed in association with esophagitis (4). However, IL-6, an important antiapoptotic cytokine, has not previously been studied in relation to BE.

*Fig. 6* Immunohistochemical staining patterns for the interleukin-6/signal transducer and activator of transcription 3 signaling pathway in different tissues from one individual patient. The intense staining of interleukin-6, phosphorylated signal transducer and activator of transcription 3, Bcl-xL, and Mcl-1 was observed in the epithelial cells of Barrett’s esophagus, whereas less intense staining was detected in duodenum and adjacent and distant squamous epithelium.
In the present report, we have shown for the first time that BE tissues secrete significant amounts of IL-6 compared with duodenum and adjacent and nonadjacent squamous epithelium. Our studies using immunohistochemistry, protein array, and real-time reverse transcription-PCR showed elevated expression of IL-6 and IL-6 mRNA in BE mucosa. In addition, we found that phosphorylated STAT3, a transcription factor that is activated by IL-6, is expressed in BE tissues at higher levels than in duodenum and squamous epithelium. Two antiapoptotic proteins associated with IL-6 signaling, Bcl-x_L and Mcl-1, were also expressed in BE tissue. The increase in IL-6, Bcl-x_L, and Mcl-1 may be related to the development of apoptosis resistance, a characteristic of BE tissue (33, 34).

The acquisition of apoptosis resistance has been closely linked to the development of cancer (5, 35). However, only a few studies have been performed to evaluate the expression of apoptotic makers in preneoplastic lesion associated with esophageal ADCA (33, 34). Several authors (33, 34) recently reported that the apoptotic balance in the transformation from intestinal metaplasia to ADCA switches to an antiapoptotic phenotype because of increased Bcl-x_L expression and decreased Bax expression. We have stressed BE tissue ex vivo with a multiple stress inducer, deoxycholate, and found that the epithelial cells including the goblet cells of BE tissue are extremely resistant to apoptosis compared with normal colonic mucosa (data not shown).

IL-6 activity is mediated through activation of three different pathways: (a) IL-6 induces association of signal transducer gp130 and ErbB, which leads to the activation of the mitogen-activated protein kinase pathway and activation of transcription factor nuclear factor-IL-6; (b) IL-6 also promotes activation of phosphatidylinositol 3′-kinase, a prominent kinase associated with apoptosis resistance (36); and (c) IL-6 signaling is mediated primarily by the Janus-activated kinase/STAT pathway. In

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**Fig. 7** Summary of all immunohistochemical experiments for the interleukin-6/signal transducer and activator of transcription 3 signaling pathway in different tissues. Expression pattern of individual patient biopsies from 10 patients, in whom all four markers were available (IL-6, phosphorylated signal transducer and activator of transcription 3, Bcl-x_L, and Mcl-1). The scoring used a simple grading system of 0–3. Overall staining was evaluated in biopsies in duodenum (DUOD), Barrett’s esophagus (BE), and squamous epithelium close to BE (SQAD) and away from BE (SQ AW). Median values are shown.
this pathway, IL-6 binds to either cognate IL-6 receptor (IL-6Ra) or sIL-6R. The complex of IL-6 and its receptor then interacts with membrane-bound signal transducer gp130 (37). This event leads to the phosphorylation of Janus-activated kinases and subsequent phosphorylation of transcription factor STAT3. Activated STAT3 then forms dimers and translocates from the cytoplasm to the nucleus. In the nucleus, STAT3 activates the transcription of specific genes by binding to consensus DNA elements. IL-6-induced STAT3 activation leads to the increased expression of antiapoptotic and angiogenic genes, including Bcl-xL, Mcl-1, angiogenin, and vascular endothelial growth factor (14, 15, 17, 38, 39).

Our findings on BE and one case of esophageal ADCA are consistent with previous reports that have shown increased IL-6 expression in various cancers (19, 21, 25, 28, 29). It has been speculated that IL-6 plays a role in the development of apoptosis resistance and angiogenesis and thus enhances tumor progression and metastasis formation (13, 14, 16, 17, 39). In normal cells, IL-6 and STAT3-mediated gene expression is transient and tightly regulated. In contrast, constitutive activation of STAT3 is linked to up-regulation in the expression of Mcl-1 and Bcl-xL, antiapoptotic members of the Bcl-2 family (14, 15, 31, 40, 41). In esophageal ADCA, expression of several antiapoptotic genes was recently evaluated. For example, Soslow et al. (34) reported significantly elevated levels of Bcl-xL in BE, whereas no correlation was found for antiapoptotic Bcl-2. The increase in Bcl-xL correlated with the degree of dysplasia being the highest in ADCA. In another study, van der Woude et al. (33) reported increased Bcl-xL expression in BE. However, no reported studies have addressed the possible mechanism of Bcl-xL up-regulation or evaluated Mcl-1 expression in BE. We speculated that an important pathway involved in the up-regulation of antiapoptotic and angiogenic genes, including Bcl-xL, Mcl-1, angiogenin, and vascular endothelial growth factor (14, 15, 17, 38, 39).

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