Published in final edited form as:

Am J Gastroenterol. 2009 February ; 104(2): 302–309. doi:10.1038/ajg.2008.85.

Expression of Bile Acid Transporting Proteins in Barrett's Esophagus and Esophageal Adenocarcinoma

Katerina Dvorak, PhD^{1,2,3}, George S. Watts, PhD^{2,4}, Lois Ramsey, BS³, Hana Holubec, MS¹, Claire M. Payne, PhD^{1,2}, Carol Bernstein, PhD^{1,3}, Gareth J. Jenkins, PhD⁵, Richard E. Sampliner, MD^{2,6}, Anil Prasad, MD⁷, Harinder S. Garewal, MD, PhD^{2,3,8}, and Harris Bernstein, PhD^{1,2}

¹Department of Cell Biology and Anatomy, The University of Arizona, Tucson, Arizona, USA

²Arizona Cancer Center, The University of Arizona, Tucson, Arizona, USA

³Section of Hematology/Oncology, Southern Arizona VA Health Care System, Tucson, Arizona, USA

⁴Department of Pharmacology, The University of Arizona, Tucson, Arizona, USA

⁵Institute of Life Science, Swansea School of Medicine, Swansea University, Swansea, UK

⁶Section of Gastroenterology, Southern Arizona VA Health Care System, Tucson, Arizona, USA

⁷Section of Pathology, Southern Arizona VA Health Care System, Tucson, Arizona, USA

⁸Department of Internal Medicine, College of Medicine, The University of Arizona, Tucson, Arizona, USA

Abstract

OBJECTIVES—Barrett's esophagus (BE) is a metaplastic lesion characterized by replacement of the normal squamous epithelium by columnar intestinal epithelium containing goblet cells. It is speculated that this process is an adaptation to protect cells from components of refluxate, such as gastric acid and bile acids. In contrast to the normal squamous epithelium, enterocytes of the distal ileum are adapted to transport bile acids from the intestinal lumen. Several bile acid transporters are utilized for effective removal of bile acids, including the apical sodium-dependent bile acid transporter (ASBT), the ileal bile acid-binding protein (IBABP), and the multidrug-resistant protein 3 (MRP3). We hypothesized that one of the possible functions of newly arising metaplastic epithelium, in the esophagus, is to transport bile acids. Our major goal was to evaluate the

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Correspondence: Katerina Dvorak, PhD, Department of Cell Biology and Anatomy, University of Arizona, 1501 N. Campbell Avenue, P.O. Box 245044, Tucson, Arizona 85724, USA. kdvorak@email.arizona.edu.

Specific author contributions: Katerina Dvorak developed the idea, designed the experiment, wrote the majority of the paper, and sponsored the project; Lois Ramsey performed immunohistochemical staining and evaluated slides; Hana Holubec evaluated slides and reviewed paper; Anil Prasad performed pathological evaluations; George S. Watts performed the RT-PCR study, evaluated RT-PCR data, and wrote the section on RT-PCR; Gareth J. Jenkins performed RT-PCR, evaluated RT-PCR data, and reviewed the paper; Harinder Garewal read and reviewed the paper; Carol Bernstein evaluated data and wrote part of the discussion; Claire M. Payne evaluated data and wrote part of the discussion; Richard E. Sampliner collected the majority of tissues and critically reviewed the paper; Harris Bernstein evaluated data and wrote part of the paper.

Potential competing interests: None.

expression of bile acid transporters in normal squamous epithelium, BE with different grades of dysplasia, and esophageal adenocarcinoma (EAC).

METHODS—A total of 101 patients were included in this study. Immunohistochemistry (IHC) and reverse transcriptase (RT)–PCR were used to detect the expression of these transporters at the mRNA and protein levels.

RESULTS—Our immunohistochemical studies showed that all three bile acid transporters are expressed in BE glands, but not in squamous epithelium. ASBT was found in the apical border in BE biopsies. The highest frequency of ASBT expression was in patients with nondysplastic BE (9 of 15, 60%), and a progressive loss of ASBT was observed through the stages of dysplasia. ASBT was not detected in EAC (0 of 15). IBABP staining was observed in the cytoplasm of BE epithelial surface cells. Expression of IBABP was found in 100% of nondysplastic BE (14 of 14), in 93% of low-grade dysplasia (LGD, 15 of 16), in 73% of high-grade dysplasia (HGD, 10 of 14), and in 33% of EAC (5 of 15). MRP3 was expressed in the basolateral membrane in 93% of nondysplastic BE (13 of 14), in 60% of LGD (10 of 16), and in 86% of HGD (11 of 13). Only weak MRP3 staining was detected in EAC biopsies (5 of 15, 33%). In addition, RT–PCR studies showed increased expression of mRNA coding for ASBT (6.1×), IBABP (9.1×), and MRP3 (2.4×) in BE ($N = 13$) compared with normal squamous epithelium ($N = 15$). Significantly increased mRNA levels of IBABP (10.1×) and MRP3 (2.5×) were also detected in EAC ($N = 21$) compared with normal squamous epithelium.

CONCLUSIONS—We found that bile acid transporters expression is increased in BE tissue at the mRNA and protein levels and that expression of bile acid transporter proteins decreased with progression to cancer.

INTRODUCTION

Barrett's esophagus (BE) is a common lesion of the distal esophagus affecting 1.6% of the general population in the western world, primarily individuals with chronic gastroesophageal reflux disease (1,2). It is a condition where squamous epithelial cells are replaced by metaplastic columnar epithelial tissue containing goblet cells. The metaplastic tissue that characterizes BE is associated with increased risk of esophageal adenocarcinoma (EAC), a cancer that has a poor prognosis with a median survival of less than 1 year (3–5).

Epidemiological and clinical studies support the hypothesis that BE results from chronic exposure of the lower esophagus to gastric acid, proteases, and bile acids (6). Importantly, individuals with BE have a higher exposure to bile acids than normal individuals or those with mild esophagitis (7,8), and BE patients with early adenocarcinoma have a higher exposure to bile acids than individuals with nondysplastic BE (9). Although bile acids are critical for digestion and absorption of fats and fat-soluble vitamins in the small intestine, chronic exposure of the distal esophagus to hydrophobic bile acids during reflux can be damaging to cells (10). At the increased concentrations accompanying a high-fat diet, bile acids affect epithelial function and integrity by triggering various signaling pathways and processes including induction of apoptosis, mitochondrial alterations, oxidative/nitrosative stress, DNA damage, and mutations (11–16). Furthermore, the combination of cytotoxic bile

acids and gastric acid induces epithelial damage and activates stress-response pathways (17–20).

In contrast to the normal squamous esophageal cells, enterocytes of the distal ileum are adapted to transport bile acids from the intestinal lumen back to the bloodstream (21). Normally, large amounts of bile acids are secreted into the intestine every day, but only relatively small quantities are lost from the body. Several different bile acid transporters are used for effective removal of bile acids from the lumen and from the cytosol of the enterocytes (22). Bile acids are transported from the apical surface into the enterocytes by the apical sodium-dependent bile acid transporter (ASBT) also referred to as IBAT (ileal bile acid transporter) (23–25). Bile acids are then bound to the ileal bile acid-binding protein (IBABP), transported across the cell to the basolateral membrane, and exported from the cells by the multidrug-resistant protein 3 (MRP3) or the heteromeric organic solute transporter (OST α/β) (21,22).

The major aim of this study was to evaluate the expression of specific bile acid transporters in BE tissue, with various degrees of dysplasia, and in EAC. Clinical and epidemiological studies suggest that the replacement of squamous epithelium (SQ) by intestinal-type epithelium is an adaptive mechanism to protect cells from components of refluxate. Thus, we hypothesize that one of the possible functions of newly arising metaplastic intestinal epithelium, in the esophagus, is to transport bile acids. Our results support this hypothesis and also indicate that this ability to transport bile acids is gradually reduced or lost as BE progresses to adenocarcinoma.

METHODS

Patient biopsies

A total of 101 patients with nondysplastic BE, various degrees of BE dysplasia or EAC were included in this study. The samples of BE, EAC, and control tissues (squamous mucosa) were taken from patients with BE and EAC who were undergoing surveillance endoscopy or surgery. **Table 1** indicates the age, gender, length of BE, degree of dysplasia or the presence of EAC with differentiation status, and experimental method used for each patient. The nondysplastic group of BE patients included 26 men and no women, their median age was 69 years (range 44–81 years) and their median BE length was 5 cm (range 1.5–11 cm). Patients with low-grade dysplasia (LGD) included 18 men and 1 woman, their median age was 72 years (range 47–81 years) and their median length of BE was 6 cm (< 3–11 cm). A total of 17 patients (all men) had BE with high-grade dysplasia (HGD), their median age was 68 years (range 41–77 years) and their median BE length was 5 cm (range 2.5–10 cm). Our study also included 37 patients with EAC (36 men and 1 woman), whose median age was 74 years (range 52–89 years). Altogether, there were 99 men and two women included in this study. They had given written informed consent as approved by the local institutional Human Subjects Committee. Endoscopic biopsies were taken from patients using a therapeutic endoscope and a large capacity biopsy forceps and immediately fixed in 10% buffered formalin or transferred to 2 ml of RNAlater solution. BE was defined histologically as the presence of intestinal-like metaplastic epithelium containing goblet cells. BE biopsies

were stained with hematoxylin and eosin and Alcian blue (pH 2.5) for goblet cells and the degree of dysplasia was evaluated by two expert pathologists.

Real-time RT–PCR

Total RNA was isolated from biopsies of normal SQ, BE, and EAC from 42 patients. Real-time reverse transcriptase (RT)–PCR was employed to quantify relative mRNA levels of MRP3, IBABP, and ASBT, as described earlier (26). Briefly, after the RT of total RNA, cDNA was amplified using TaqMan primer/probes specific for GAPDH, MRP3, IBABP, and ASBT obtained from Applied Biosystems TaqMan probe collection (Applied Biosystems, Foster City, CA). The primer probe sequences are available upon request. Real-time PCR was carried out on 5 ng of cDNA using parameters and reagents recommended by Applied Biosystems on an Applied Biosystems 7500 real-time PCR system. GAPDH expression was used to control for technical variation and relative mRNA levels were quantitated using the comparative C_t method, as described earlier (26). Relative expression values were $\log_2$ transformed and normalized so that the mean of the normal samples was one. Statistically significant differences were evaluated using a two-tailed Student's *t*-test for heterostochastic unpaired samples. Expression values were graphed in a boxplot using a utility written for Microsoft Excel (freely available at: <http://peltiertech.com/Excel/Charts/BoxWhisker.html>).

Immunohistochemical staining

For the evaluation of ASBT and IBABP in formalin-fixed, paraffin-embedded tissues, standard immunohistochemical assays involving biotin–avidin linked peroxidase detection were used, as described earlier (20,27). Briefly, paraffin-embedded sections were deparaffinized, rehydrated, and placed in 0.5% hydrogen peroxide for 30 min to block endogenous peroxidase. Antigen retrieval was performed by immersing slides in 0.2% saponin (Sigma, St Louis, MO; S-7900) for 30 min. Slides were then incubated for 60 min with 1.5% normal goat serum for ASBT or 1.5% normal rabbit serum for IBABP, followed by overnight incubation at 4 °C with anti-ASBT, rabbit polyclonal antibody (1.0 μ g/ml, a generous gift from Paul Dawson, Wake Forest University Baptist Medical Center, Winston-Salem, NC) or with anti-ILBP, goat polyclonal antibody (1.0 μ g/ml, SC-23994; Santa Cruz Biotechnology, Santa Cruz, CA). Negative controls (pooled normal rabbit IgG or normal goat IgG) were run for each slide. After three rinses with phosphate-buffered saline, the secondary antibody (biotinylated goat anti-rabbit IgG, or rabbit anti-goat IgG, 1:400; Vector Laboratories, Burlingame, CA) was applied for 30 min. Slides were again rinsed with PBS and Vectastain ABC reagent (Elite PK-6100, Standard, Vector Laboratories) was added according to the kit instructions for 30 min. After three final phosphate-buffered saline rinses, slides were immersed in 3,3'-diaminobenzidine, at a concentration of 0.25 mg/ml, activated with hydrogen peroxide for 5 min, rinsed, and lightly counterstained with hematoxylin.

A similar method was used to detect MR3P (3 μ g/ml, anti-MR3P, Clone M3II-9, monoclonal antibody; Kamiya Biomedical, Seattle, WA). However, here, antigen retrieval was performed using a microwave protocol, and Tris-buffered saline Tween-20 was used as the wash and dilution buffer. Slides were then incubated for 60 min with 5% normal horse

serum followed by overnight incubation at 4 °C with anti-MR3P antibody. Negative controls of normal mouse IgG1 were run simultaneously.

Evaluation of immunohistochemical staining

Immunohistochemical staining was independently scored by three experienced histologists using a Nikon Eclipse E400 brightfield microscope equipped with a digital camera and Image-Pro software. The pattern (apical membrane, cytoplasm, basolateral membrane) and extent of staining of each tissue was evaluated and the percentage of positively stained biopsies in each category was assessed. Each slide was compared with its negative control (we included a negative control for each slide stained with anti-ASBT and anti-IBABP polyclonal antibody). As anti-MRP3 antibody is a monoclonal antibody and negative controls were completely clean we did not have negative control slide for each tested slide. We looked at all sections that were on the slide, usually we had 3–4 sections per slide. Scoring was simplified to yes or no for each biopsy, as the staining was either present or absent and there were no systematic differences in the intensities of staining.

RESULTS

ASBT is expressed in the apical membrane of BE biopsies, but not in squamous epithelium

ASBT is a bile acid transporter that facilitates transport of bile acid from the lumen across the apical border. A total of 59 biopsies from four groups with different grades of dysplasia (**Table 2**; 15 nondysplastic (ND), 15 LGD, 13 HGD, 16 EAC) along with SQ biopsies were evaluated for ASBT expression by standard immunohistochemical staining. ASBT was focally expressed in the apical border in BE biopsies (**Figure 1**). No staining of ASBT was found in the apical region of SQ (**Figure 1**). The highest frequency of expression of this transporter was observed in nondysplastic BE tissues (9 of 15, 60%) and a progressive loss of ASBT was observed through the stages of dysplasia (**Table 2**). In LGD, 8 of 15 (53%) of tissues stained positively and in HGD, 4 of 13 (31%) of tissues stained for ASBT. ASBT was not detected in the adenocarcinoma tissues (0 of 16, 0%).

IBABP is expressed in the cytoplasm of BE biopsies

IBABP binds to bile acids and transports them through the cytoplasm to the basolateral membrane. We evaluated 15 BE biopsies in each of the four groups with different grades of dysplasia along with SQ. Although no staining was detected in SQ, distinct and consistent staining was observed in the cytoplasm of epithelial surface cells of BE tissue (**Figure 1**). Expression of IBABP was found in nondysplastic BE tissue (14 of 14, 100%). IBABP was also detected in LGD samples (15 of 16, 94%) and HGD biopsies (10 of 14, 71%), whereas only 33% of EAC biopsies (5 of 15) stained positively with IBABP antibody.

MRP3 is expressed in the basolateral membrane in BE biopsies

MRP3 was strongly expressed in the basolateral membrane in nondysplastic BE as well as in LGD and HGD (**Figure 1**). No staining was observed in the negative control (**Figure 1**) or in SQ taken 5 cm away from the BE tissue (**Figure 1**). Strong staining of MRP3 was found in the majority of nondysplastic biopsies (13 of 14, 93%), LGD (10 of 16, 63%), and HGD

(11 of 13, 85%). Only weak, focal staining for MRP3 was detected in biopsies from cancer patients (5 of 15, 33%) and no staining for MRP3 was detected in the remaining 10 of 15 (67%) of adenocarcinoma biopsies.

mRNA levels of bile acid transporters are elevated in BE compared with squamous epithelium

In addition to the samples obtained for immunohistochemical staining, 49 samples were obtained for mRNA studies. The tissues evaluated included 13 BE (nine samples were from patients with nondysplastic BE, two samples were from patients with focal LGD and two from patients with HGD), 15 normal squamous epithelia, and 21 EAC. The mRNA expression of all three bile acid transporters was significantly increased in BE tissues compared with SQ (**Figure 2**). The relative mean expression of ASBT mRNA in BE was 6.07 ± 0.14 (s.e.m.) compared with SQ (1.0 ± 0.26 , $P < 0.001$). The relative mean level of IBABP mRNA in BE was 9.15 ± 0.32 compared with SQ (1.0 ± 0.56 ; $P < 0.001$) and the relative mean levels of MRP3 mRNA in BE were 2.39 ± 0.01 compared with SQ 1.0 ± 0.03 ; $P < 0.001$). The relative expression mean mRNA levels of IBABP and MRP3 were also significantly increased in EAC samples compared with SQ. The increase in ASBT mRNA levels in EAC compared with SQ was not statistically significant due to high variability. The relative mean levels of mRNA coding for ASBT, IBABP, and MRP3 in EAC were 2.65 ± 0.44 ($P > 0.05$), 10.1 ± 2.1 ($P < 0.01$), and 2.49 ± 0.02 ($P < 0.001$), respectively (**Figure 2**). In addition, we observed a significant decrease in ASBT Expression ($P < 0.05$) when mRNA levels of the EAC samples were compared with the BE tissues (**Figure 2**). No significant differences for IBABP and MRP3 ($P > 0.05$) were detected in EAC compared with BE.

DISCUSSION

We report here that bile acid transporters are expressed in BE tissues, especially in nondysplastic and low-grade dysplastic BE tissues. This finding provides further support for the concept that BE arises in response to stresses caused by elevated exposure to bile acids during frequent reflux episodes. It also suggests that development of BE is an adaptive response to this stress. Our findings also indicate that as BE progresses towards a more advanced disease, the expression of these transporters declines. Although we found expression of ASBT, IBABP and MRP3 in the ND and LGD stages of the disease, there was lower or no expression at the protein level of the three bile acid transporters, ASBT, IBABP, or MRP3, in adenocarcinomas. In BE tissue, ASBT was localized to the apical membrane, IBABP was confined to the cytoplasm, and MRP3 was present in the basolateral membrane (**Figure 1**). In our studies, we chose immunohistochemistry (IHC) as a method for detection and histological localization of bile acid transporters in different tissues, as western blot analysis does not allow one to distinguish where the individual transporters are expressed and/or whether the evaluated tissue is truly BE or a combination of SQ and BE.

We also evaluated mRNA levels of these three bile acid transporters. Importantly, we found significantly increased mRNA levels of all these proteins in BE tissue compared with SQ. These data are consistent with our immunohistochemical studies. Increased levels of mRNA

coding for ASBT, IBABP, and MRP3 were detected in EAC compared with SQ. No significant differences for mRNA of IBABP and MRP3 were detected in EAC compared with BE. However, we found significantly decreased levels of mRNA coding for ASBT in EAC compared with BE tissue, which is also consistent with our studies using IHC.

Normally, most of the bile acids delivered into the duodenum are re-absorbed in the ileum through the action of the transporters, apical ASBT, cytosolic IBABP, and basolateral MRP3/OST α/β (21). Transcriptional regulation of these bile acid transporters is closely related to the regulation of lipid and cholesterol homeostasis (28). Human ASBT is a 39-kDa protein that transports conjugated and unconjugated bile acids with high efficiency and is abundantly expressed in the ileum (29). ASBT is also expressed in renal tubule cells, cholangiocytes, and the gallbladder (22,28). IBABP is a 14-kDa protein that binds to bile acids and is expressed in the cytoplasm of epithelial cells of the ileum. The transcription factor responsible for the regulation of IBABP expression is farnesoid X receptor (FXR). Importantly, De Gottardi *et al.* (30) reported that FXR is detected in nondysplastic BE, but not found in normal esophageal mucosa, esophagitis, and EAC. Similar results were obtained by Mekkala *et al.* (31) indicating that FXR is expressed in BE, but not in EAC. In addition, a recent study by Capello *et al.* (32) have shown that mRNA of FXR and IBABP are elevated in BE compared with SQ and that deoxycholic acid (DCA) can increase FXR and IBABP mRNA levels in the TE7 esophageal cell line. Altogether, these data are consistent with our results indicating that IBABP expression is elevated in BE, whereas decreased in EAC and absent in normal SQ. MRP3 is active in the transport of the mono-anionic human bile constituent glycocholate (33).

During reflux episodes, the normal SQ is exposed to both gastric acid and duodenal secretions containing bile acids (8,34–37). Hydrophobic bile acids are thought to be important in the development of gastrointestinal malignancies of epithelial origin (16). In humans, the incidence of cancers of the laryngopharyngeal tract, esophagus, stomach, pancreas, small intestine (near the ampulla of Vater), and colon are all positively associated with intestinal levels of bile acids (16). In patients with esophagitis, BE, and EAC pathologic exposure to duodenal refluxate was measured by bilirubin monitoring using a Bilitec probe (9). Evidence for pathologic exposure was observed in 22.2% of patients with esophagitis, 54.5% of patients with BE, and 78.6% of patients with EAC, suggesting a role for bile acids in EAC pathogenesis. An analysis of the esophageal aspirates of gastroesophageal reflux disease patients showed that bile acids, mostly glycine conjugates, are present in 86% of patients (38). Kauer *et al.* (38) reported that the bile acids present in refluxate of patients with gastroesophageal reflux disease are glycocholic acid (60%), glycodeoxycholic acid (16%), glycochenodeoxycholic acid (15%), and a mixture of taurine conjugated bile acids (9%). Nehra *et al.* (8) found that DCA and taurocholic acid are also present in the refluxate of BE patients. More toxic unconjugated bile acids, such as DCA, are normally formed by the action of colonic bacteria. Interestingly, Theisen *et al.* (39) reported that patients taking proton pump inhibitors have a significantly higher incidence of gastric bacterial overgrowth and consequently increased concentrations of unconjugated bile acids, including DCA. Therefore, the decrease in expression of bile acid transporters that we

observed during progression of BE to EAC may be responsible, in part, for increased cellular damage resulting from an increase in bile acid levels.

In order for carcinogenesis to occur, mutations must be produced. DCA has been shown to induce chromosomal instability in esophageal cells using a micronucleus cytokinesis assay (40). Components of the duodeno-esophageal refluxate are mutagenic, as shown by *in vivo* experiments using Big Blue rats (41). This may explain why duodeno-esophageal reflux induces EAC without an exogenous carcinogen in surgical animal models of BE and EAC (42). Another study showed that the typical injuries and cellular changes seen in severe reflux esophagitis that may lead to the development of BE, are induced in rats by continuous perfusion with bovine bile after treatment for only 4 weeks (43). These results underscore the importance of bile acids in BE as a premalignant lesion.

Metaplasia is the replacement of one cell type with another cell type. Such a replacement is generally caused by an abnormal stimulus because of damage and/or chronic inflammation. We speculate that esophageal stem cells, if exposed to noxious agents causing injury or inflammation, can be reprogrammed to form a specialized tissue that is resistant to these noxious agents. As BE resembles intestinal tissue, it is possible that the formation of this metaplastic tissue is an adaptive response to bile acids present in refluxate. However, this may not be a sufficient protective mechanism against repeated exposures to bile acids. The concentrations of bile acids in the refluxate of patients with early adenocarcinoma are higher than in the refluxate of patients with uncomplicated, nondysplastic BE (9). Exposure to high bile acid concentrations may lead to saturation of the bile acid transport capacity and also an increase in passive diffusion of cytotoxic bile acids through cellular membranes (23). When high levels of bile acids are present in refluxate, bile acids may accumulate in BE cells and cause excessive cellular damage such as the induction of reactive oxygen species and associated-oxidative DNA damage, leading to dysplasia and progression to EAC. However, this hypothesis needs to be studied further as well as the mechanism by which expression of these bile acid transporters is reduced during progression from BE to EAC. Perhaps, inflammatory cytokines, including interleukin 6, a cytokine reported to be increased in BE (44), can modulate the expression of bile acid transporters (45,46). The decline in transporter expression that occurs in progression of nondysplastic BE to EAC may be a further adaptation to high bile acid exposure in order to limit DNA damage to the metaplastic cells. However, as the majority of the EACs included in our study were poorly or moderately differentiated tumors, no conclusion can be made at this point concerning association of bile acid transporter expression with the degree of differentiation.

In summary, our findings suggest that bile acids transporting proteins are expressed in BE. Future studies are warranted to explore the significance of these findings.

ACKNOWLEDGMENTS

These studies were supported by grants from the Arizona Biomedical Research Commission (no. 0012) and a SPORE grant from the National Institute of Health, Bethesda, MD (IP50 CA95060).

Guarantor of the article: Katerina Dvorak, PhD.

Financial support: We received support from ABRC (Arizona Biomedical Research Commission) 0012 (K.D.—PI) and SPORE grant (K.D., H.S.G., R.E.S.—PI project 2).

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ No studies have previously been performed on the expression of bile acid transporters in BE.

WHAT IS NEW HERE

- ✓ Expression levels of three major bile acid transporters, apical sodium-dependent bile acid transporter (ASBT), IBABP, and multidrug resistance-associated protein 3 (MRP3), were evaluated in normal SQ, in BE tissues with different grades of dysplasia, and in EAC by immunohistochemistry and real-time RT-PCR.
- ✓ Expression levels of mRNA coding for the three bile acid transporters are significantly increased in BE tissue compared with SQ.
- ✓ All three proteins associated with transport of bile acids are expressed in BE tissue, but not in SQ. In BE tissue, ASBT was localized to the apical membrane, IBABP was confined to the cytoplasm, and MRP3 was present in the basolateral membrane.
- ✓ There was a progressive loss of expression of the bile acid transporter proteins, so that expression followed the pattern: nondysplastic BE > dysplastic BE > adenocarcinoma.

Figure 1.

Evaluation of bile acid transporter proteins, ASBT, IBABP and MRP3, in Barrett's esophagus (BE), esophageal adenocarcinoma (EAC), and squamous epithelium (SQ) by immunohistochemistry. Matched BE esophagus negative controls (BE/NC) are also shown, in which rabbit or mouse IgG was added instead of primary antibody. The magnification used was $\times 1,000$ for top three images and $\times 400$ for all other images.

Figure 2.

Evaluation of ASBT, IBABP, and MRP3 mRNA expression in squamous epithelium (SQ, $N = 15$), Barrett's esophagus (BE, $N = 13$), and esophageal adenocarcinoma (EAC, $N = 21$) by real-time quantitative RT-PCR. Boxplots show median (white band), mean (red diamond), 25th and 75th percentiles (bounded by gray box), 1.5 times the interquartile range (whiskers), outliers within 3 times the first of third interquartile range (asterisk), and extreme outliers lying more than 3 times the first of third interquartile range (o). Significant differences are shown by horizontal bars with the respective P values shown.

Table 1

Patient characteristics

No.	Age	Gender	BE (cm)	Sample	E	No.	Age	Gender	BE (cm)	Sample	E
1	69	M	1.5	ND	IHC	52	59	M	NA	HGD	IHC
2	75	M	4	ND	IHC	53	76	M	4	HGD	IHC
3	70	M	7	ND	IHC	54	43	M	3	HGD	IHC
4	70	M	<3	ND	IHC	55	41	M	6	HGD	IHC
5	56	M	11	ND	IHC	56	68	M	NA	HGD	IHC
6	79	M	7.5	ND	IHC	57	59	M	NA	HGD	IHC
7	57	M	<3	ND	IHC	58	58	M	8	HGD	IHC
8	51	M	<3	ND	IHC	59	64	M	NA	HGD	IHC
9	64	M	<3	ND	IHC	60	75	M	5	HGD	IHC
10	50	M	9	ND	IHC	61	70	M	5	HGD	PCR
11	69	M	2	ND	IHC	62	72	M	2.5	HGD	PCR
12	81	M	8	ND	IHC	63	79	M	—	EAC/MP	IHC
13	66	M	4	ND	IHC	64	66	M	—	EAC/W	IHC
14	44	M	<3	ND	IHC	65	75	M	—	EAC/M	IHC
15	79	M	6	ND	IHC	66	77	M	—	EAC/M	IHC
16	50	M	5	ND	IHC	67	69	M	—	EAC/P	IHC
17	55	M	9	ND	IHC	68	70	M	—	EAC/P	IHC
18	63	M	2	ND	PCR	69	64	M	—	EAC/M	IHC
19	73	M	2.5	ND	PCR	70	76	M	—	EAC/P	IHC

No.	Age	Gender	BE (cm)	Sample	E	No.	Age	Gender	BE (cm)	Sample	E
20	73	M	4	ND	PCR	71	72	M	—	EAC/M	IHC
21	77	M	8	ND	PCR	72	73	M	—	EAC/M	IHC
22	70	M	10	ND	PCR	73	60	M	—	EAC/W	IHC
23	68	M	2	ND	PCR	74	72	M	—	EAC/M	IHC
24	71	M	5	ND	PCR	75	89	M	—	EAC/MP	IHC
25	44	M	3	ND	PCR	76	52	M	—	EAC/P	IHC
26	79	M	7	ND	PCR	77	68	M	—	EAC/P	IHC
27	52	M	<3	LGD	IHC	78	76	M	—	EAC/M	IHC
28	68	M	5	LGD	IHC	79	82	M	—	EAC/MP	PCR
29	74	M	<3	LGD	IHC	80	75	M	—	EAC/MP	PCR
30	65	M	3	LGD	IHC	81	52	F	—	EAC/P	PCR
31	77	M	<3	LGD	IHC	82	63	M	—	EAC/P	PCR
32	79	M	10	LGD	IHC	83	76	M	—	EAC/P	PCR
33	59	M	<3	LGD	IHC	84	74	M	—	EAC/MP	PCR
34	72	M	5	LGD	IHC	85	75	M	—	EAC/WM	PCR
35	81	M	11	LGD	IHC	86	65	M	—	EAC/P	PCR
36	62	M	5	LGD	IHC	87	70	M	—	EAC/W	PCR
37	79	M	6	LGD	IHC	88	81	M	—	EAC/M	PCR
38	64	M	<3	LGD	IHC	89	NA	M	—	EAC/NA	PCR
39	73	M	10	LGD	IHC	90	58	M	—	EAC/NA	PCR
40	60	M	5	LGD	IHC	91	60	M	—	EAC/M	PCR

No.	Age	Gender	BE (cm)	Sample	E	No.	Age	Gender	BE (cm)	Sample	E
41	47	M	7	LGD	IHC	92	66	M	—	EAC/M	PCR
42	61	M	6	LGD	IHC	93	46	M	—	EAC/M	PCR
43	75	M	5	LGD	IHC	94	57	M	—	EAC/P	PCR
44	80	M	5	LGD	PCR	95	45	M	—	EAC/P	PCR
45	78	F	9	LGD	PCR	96	73	M	—	EAC/M	PCR
46	72	M	2.5	HGD	IHC	97	70	M	—	EAC/NA	PCR
47	52	M	NA	HGD	IHC	98	67	M	—	EAC/P	PCR
48	75	M	5	HGD	IHC	99	63	M	—	EAC/PM	PCR
49	77	M	<3	HGD	IHC	100	80	M	—	SQ	PCR
50	68	M	NA	HGD	IHC	101	61	M	—	SQ	PCR
51	47	M	10	HGD	IHC						

M, male; F, female; E, experiment; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; IHC, immunohistochemistry; LGD, low-grade dysplasia; M, moderately differentiated; MP, moderately to poorly differentiated; NA, not available (some data are not available for archival tissues); ND, nondysplastic; P, poorly differentiated; W, well differentiated; WM, well to moderately differentiated; —, not applicable.

Table 2

The expression of bile acid transporters in esophageal tissues with different grades of dysplasia

Expression of specific bile acid transporters in esophageal tissues					
	ND	LGD	HGD	EAC	SQ
ASBT	9/15 (60%)	8/15 (53%)	4/13 (31%)	0/16 (0%)	0/5 (0%)
IBABP	14/14 (100%)	15/16 (94%)	10/14 (71%)	5/15 (33%)	0/5 (0%)
MRP3	13/14 (93%)	10/16 (63%)	11/13 (87%)	5/15 (33%)	0/5 (0%)

EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia BE; LGD, low-grade dysplasia BE; ND, nondysplastic BE; SQ, squamous epithelium.