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Relationships of CDXs and apical sodium-dependent bile acid transporter in Barrett's esophagus

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Abstract

Barrett's esophagus (BE) is characterized by intestinal metaplasia with the differentiated epithelium replaced by another type of epithelium morphologically similar to normal intestinal epithelium. The metaplasia is preceded by bile and acid reflux into the esophagus. BE is a premalignant condition associated with increased risk of esophageal cancer, especially esophageal adenocarcinoma. The Caudal-related homeodomain transcription factors Caudal-related homeodomain transcription factor CDX1 and CDX2 are expressed exclusively in the small and large intestine, playing important roles in proliferation and differentiation of intestinal epithelial cells. Ectopic expression of CDX1 and CDX2 occurs in BE. The apical sodium-dependent bile acid transporter (ASBT) is expressed primarily in terminal ileum where it is a key factor for intestinal reabsorption of bile salts. In addition to upregulation of CDX1 and CDX2, ASBT expression is up-regulated in BE. Furthermore, both CDX1/CDX2 and ASBT expressions are down-regulated in high-grade esophageal dysplasia. The alteration of the above-mentioned factors calls for attention: what is the relationship between CDXs and ASBT aberrant expression in BE? In this commentary, we discuss this issue on basis of the recent study done by Ma *et al*.

Key words: Esophagus; Intestinal metaplasia; Caudal-related homeodomain transcription factors; Apical sodium-dependent bile acid transporter; Aberrant expression

Core tip: Aberrant co-expression of Caudal-related homeodomain transcription factors (CDXs) and apical sodium-dependent bile acid transporter (ASBT) in the epithelium of Barrett's esophagus (BE) indicates association among these factors. Acid and bile reflux induce *CDXs* gene expression and can lead to formation of BE. CDX-mediated promoter activation can lead to aberrant expression of ASBT. The BE phenotype may be better than squamous epithelium to protect against refluxed acid and bile. On the other hand the BE phenotype is associated with increased risk of esophageal adenocarcinoma (EAC). Furthermore, the decreased expressions of CDXs and ASBT in high-grade esophageal dysplasia indicate that CDXs and ASBT are inhibitory factors to the progression of EAC.

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COMMENTARY ON HOT TOPICS

Recently, an interesting study by Ma *et al*[1] demonstrated that short interfering RNA-mediated knockdown of Caudal-related homeodomain transcription factors (CDXs) resulted in reduced apical sodium-dependent bile acid transporter (ASBT) mRNA expression in intestinal cells.

CDXs strongly induced activity of the ASBT promoter of esophageal and intestinal cells. Association with ASBT expression was found for CDX1, CDX2 and hepatocyte nuclear factor-1α in Barrett's esophagus (BE) biopsies. Ma *et al*[1] concluded that CDX1 and CDX2 activate the human ASBT promoter by transcription. For the first time ASBT is added to the list of genes regulated by CDXs. We strongly recommend this paper to the readers.

BE is a clinically important disease. The human esophagus is lined by a multilayered squamous epithelium which withstands the potential damage from rapidly propelling food boluses through the esophagus and also from intermittent exposure to refluxed contents from the stomach. However, the esophageal epithelium, usually at the gastroesophageal junction, can be inflamed and injured if the esophageal epithelium chronically and repeatedly is in contact with refluxed bile and acid. This can result in intestinal metaplasia where the esophageal squamous epithelium is replaced by intestinal-type epithelium, which is the key feature of BE[2]. BE is characterized not only by the morphological intestinalization but also by changes in gene expression patterns. The intestinal specific transcription factors CDX1 and CDX2 and other intestinal proteins such as villin, sucrase isomaltase, and acidic mucins/MUC2 can be detected in human BE tissue[3]. BE is an important risk factor for esophageal adenocarcinoma (EAC)[4,5]. The molecular mechanisms related to BE are not yet fully understood. Currently, it is believed that the BE cell emerges from (1) the esophageal squamous epithelium; (2) the distal esophagus submucosal gland epithelium; (3) the proximally-migrating gastric cardia epithelium; or from (4) infiltrating bone marrow stem cells[6,7]. Hence, the mechanism of BE formation is not well understood.

ASBT is a 48-kDa transmembrane protein. At the apical membrane of ileal enterocytes, ASBT is the chief mediator of active sodium-dependent intestinal bile acid absorption[8]. The roles of ASBT on bile acid reabsorption, regulation of *ASBT* gene expression and its association with some diseases have been reviewed in detail[8-11]. ASBT is mainly expressed in the terminal ileum but is also expressed in renal tubule cells, cholangiocytes, and the gallbladder[10]. It was recently shown that the expression of ASBT is elevated in esophageal epithelial cells from BE patients whereas ASBT expression was decreased in esophageal adenocarcinoma at both mRNA and protein levels[12]. CDX1 and CDX2 are expressed exclusively in the small and large intestine, playing important roles in proliferation and differentiation of intestinal epithelial cells. The role of *CDX* genes in the gut has recently been reviewed[13]. In adults, CDX1 is expressed primarily in intestinal crypts[14,15] whereas CDX2 is expressed in the paracaecal region of the intestine. Furthermore, CDX2 is expressed relatively more in the villi than in the crypts[14]. CDX1 and CDX2 are not expressed in the normal human esophagus[16]. However, CDX1 and CDX2 levels are elevated in the epithelium of BE[16,17]. CDX2 expression has been demonstrated in all biopsy specimens from BE without and with dysplasia, and from BE-associated adenocarcinomas[18,19]. Expression of CDX1 mRNA and protein was found in biopsy specimens from patients with BE but not from epithelium of normal esophagus[20]. Furthermore, similar to expression of ASBT, CDX2 expression decreased esophageal metaplasia progressed into adenocarcinoma[21,22].

The aberrant co-expression of CDXs and ASBT in the BE epithelium makes us ask what is the relationship between CDXs and ASBT, how do these factors relate to BE, BE with dysplasia and BE-associated adenocarcinomas. In order to study the relationship between CDXs and ASBT, Ma *et al*[1] conducted a test series to (1) study whether endogenous human ASBT mRNA levels are regulated by CDX1 and CDX2; (2) study the possible direct role for CDX1 and CDX2 in the regulation of the ASBT promoter; (3) identify putative CDX response elements (CDXREs) within the ASBT; (4) study whether the proximal promoter region containing the predicted CDXREs mediate the CDX-dependent activation; (5) study the potential *in vitro* interaction between CDX1 and CDX2 with their predicted binding motifs within the ASBT promoter; and to (6) confirm the interaction between CDX1 and CDX2 with the ASBT promoter also within living cells. Ma *et al*[1] found in human esophageal and intestine-derived cell lines that the human ASBT promoter is a direct target for transcriptional activation by the transcription factors CDX1 and CDX2. In other words, ASBT expression is regulated by CDXs. Therefore, their study adds a new piece of knowledge to the already known complexity of transcriptional regulation of *ASBT* gene expression. Furthermore, Ma *et al*[1] discover close association of CDX and ASBT expression levels in human BE tissue. This indicate that CDX-mediated ASBT promoter activation can lead to aberrant esophageal expression of the bile acid uptake system ASBT and consequently to an increase in epithelial bile acid uptake activity by the BE mucosa.

It is well known that BE is closely associated with gastro-esophageal reflux disease (GERD). In animal models, GERD caused increased Cdx2 expression in cells of the basal layer of esophageal squamous epithelium. The increased Cdx2 expression preceded the development of intestinal metaplasia[23,24]. In esophageal biopsy specimens from patients with BE, Vallböhmer *et al*[17] found high levels of CDX2 mRNA in specialized intestinal metaplasia. A recent study done by Kazumori *et al*[25] shows that Cdx1 is over-expressed in esophageal metaplastic tissue and that bile acids increase promoter activity in cultured esophageal epithelial cells. This in turn appears to induce production of Cdx2 sufficient to cause intestinal metaplasia. It has been proposed that bile acids in refluxed contents cause tight junctions in squamous cells to break, allowing the bile acids to leak into the basal layer, resulting in cell transdifferentiation[26].

From the above-mentioned studies it is evident that acid reflux and bile reflux contribute to increased CDX expression levels. The induction of *CDXs* genes precedes

the morphologic changes in BE. The BE phenotype may be better than squamous epithelium to protect against exposure to refluxed acid and bile. Furthermore, CDX-mediated promoter activation leads to aberrant expression of ASBT, resulting in increased epithelial bile acid uptake by the BE mucosa. However, the BE phenotype has 30-125 times increased risk of EAC compared to that of the general population[27]. Furthermore, although CDXs expression can be detected in well or moderately well differentiated EAC, expression of CDXs is decreased and may even be undetectable in poorly differentiated EAC[28,29]. Ma *et al*[1] found that ASBT like CDXs decrease its expression in high-grade esophageal dysplasia. All together this suggests that CDXs and ASBT expression have an inhibitory role for the progression of EAC. However, the exact mechanism about the effect of CDX1, CDX2 and ASBT on BE and BE-associated esophageal dysplasia is not well understood yet and need further study.

In summary, aberrant co-expression of CDXs and ASBT in BE epithelium stimulates further interest into learning more about the relationship between CDXs and ASBT and how they relate to BE. Based on reviewing the study by Ma *et al*[1] and other relevant literature, it is anticipated that *ASBT* gene expression is regulated by CDXs. Acid and bile reflux as well as inflammation induce *CDXs* gene expression preceding BE. CDX-mediated promoter activation can lead to aberrant expression of ASBT. The BE phenotype may be better than squamous epithelium to protect against refluxed acid and bile. On the other hand, BE phenotype is associated with increased risk of EAC. Furthermore, CDXs and ASBT expressions decrease in high-grade esophageal dysplasia. This indicates that CDXs and ASBT expression may be inhibitory factors for progression of EAC. Further research is needed for understanding the exact mechanism and effects of CDX1, CDX2 and ASBT on BE and BE-associated esophageal dysplasia.

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