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# Pathogenesis of Barrett's Esophagus

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**Abstract**

Barrett's esophagus (BE) is characterized as histologic evidence of intestinal metaplasia is present in distal esophageal epithelium and is an important pathology because it is the major risk factor for developing esophageal adenocarcinoma (EAC). It's well known that chronic gastroesophageal reflux disease (GERD) leads to the development of metaplasia. However, the cellular and molecular mechanism of the replacement of squamous esophageal epithelium with a columnar type is largely unknown.

Efforts to understand the pathogenesis of BE and its disposition to EAC have been increasing over the previous 2 decades. This review aims to explore current data on the major risk factors, origin, genetic changes of BE, provide an insight into the molecular biomarkers related with BE and seek for possible development of therapies that could prevent BE from progressing to EAC.

**Keywords:** Barrett's esophagus; Pathogenesis; Cell origin; Signaling pathways; Development

## Introduction

Barrett's esophagus (BE), also termed columnar lined esophagus, is metaplasia that the esophageal non-keratinizing squamous epithelium was replaced with a columnar mucosa [1,2]. It is considered as the "cancerization field" in metaplastic epithelium, developing Barrett's adenocarcinoma [3-5]. Gastroesophageal reflux disease (GERD) is suggested as a major fisk factor of BE through repeated mucosal damage. It's well known that chronic GERD leads to the esophagitis, if persistent and recurrent, resulting in columnar metaplasia and eventually "intestinal" metaplasia. The pathogenetic mechanism of BE, particularly its origin of cell, is not clear [6]. The question of origin of the metaplastic epithelium in BE is important, not only for a better understanding of the results of present treatment modalities but also for the development of new surgical and pharmacological treatments and preventions. This review focuses on recent advances regarding the pathogenesis of BE.

## Major Risk Factors of BE

Major risk factors for the development of BE include a long-standing history of GERD (>5 years), a large hiatus hernia and other reported risk factors containing obesity, white ethnicity, alcohol consumption, tobacco use, duodenal-gastric reflux, delayed clearance of esophageal acid, defect of lower esophageal sphincter and profound reflux of gastric juice into the lower esophagus, and use of anticholinergic medications [7].

GERD is the most important etiological factor for BE. Reflux of gastric content into the esophagus has been considered answerable for the onset of BE. The major harmful components of refluxed gastric material are acid and bile salts. Both clinical and experimental studies have shown that bile acids are noxious to the esophageal mucosa. The severity of mucosal damage was increased in patients with both gastric and duodenal juice reflux, comparing with gastric juice reflux alone [8]. Bile salts, or more accurately, duodenal content, have been implicated in the pathophysiology of esophageal mucosal injury for decades. Numerous studies have shown significant effects of bile salts and other reflux components on esophageal epithelial physiology, resulted in activating of protein kinase C and nuclear transcription factors [9]. These findings, in concert with the strong link between GERD and BE or EAC suggests that bile salts play a crucial role in the pathophysiology of BE and EAC.

## Cell Origin of BE

It is well known that BE is an acquired condition secondary to symptoms of chronic gastroesophageal reflux disease (GRED), however, the cellular and molecular mechanisms involved in BE development are poorly understood. In particular, the cell origin of Barrett's esophagus is still controversial. Several theories attempt to explain the cellular origins of BE, and all these hypotheses were based on experimental and clinical evidences.

### Migration of cells from gastric cardia epithelium

Esophageal squamous epithelium transformed initially into columnar epithelium morphologically similar to that of gastric cardia, which composed of columnar epithelium with underlying mucous glands or mixed mucous/oxyntic glands, before the formation of goblet cells [6,10,11]. The promising cell source of BE was the migrating epithelium of gastric cardia, which repairing gastroesophageal reflux-mediated damage to the adjacent esophageal epithelium. Hayward et al. [12] have firstly demonstrated the possibility that BE arises from the migration of columnar epithelium of the cardia or proximal stomach up the esophagus to repair the damaged squamous epithelium due to GERD. At present, it is believed that BE develops de novo from cells intrinsic to the esophagus rather than migrating from the stomach [13].

Recently, one new mouse model in which overexpression of interleukin-1beta (IL-1β) repeats human pathology with BE with histopathology and gene profiles similar to human BE has been carried out by Quante et al. [14]. Lgr5, a marker of active stem cells in the small intestine and colon cannot be detected in normal esophagus but emerge in the gastric cardia at low level. In the model, Lgr5+ stem cells were detected in gastric cardia of BE patients, these stem cells can grow up in the distal esophagus and develop a columnar like epithelium. It is believed that inflammation induced migration of cardia progenitor cells (including Lgr5+ cells) and their metaplastic descendants into the esophagus. Gastric cardia progenitors are probably the source of BE.

## Migration upward of sub esophageal gland cells

Oppositely, several animal researches provided strong evidence that the original cell of BE probably resides in the esophagus instead of the proximal stomach [15,16]. For example, both Gillen et al. [15] and Li et al. [16] demonstrated that columnar re-epithelization may occur from cells intrinsic to the esophagus and is not dependent on proximal migration of cardia columnarepithelium. Morphologic and molecular evidences suggested that the esophageal mucosal gland ducts harbor stem cells have potential of differentiation into columnar epithelium [17,18]. Evidence supporting this theory were rooted in both morphologial and cytochemical studies.

Many reports suggested that the gastric acid and bile reflux mixture activate the esophageal stem cell to transform the intestine-type columnar epithelium. The candidate stem cells in the esophagus include stem cells contained in the duct epithelium of the superficial cardia glands, or submucosal glands of the esophagus [19-21]. Reflux mediated reprogramming of stem cell, resulting in stem cell migration from submucosal ducts or glands in order to repopulate the damaged esophageal mucosa [9,22]. However, the theory of submucosal glands has been called into question since BE developed in rat models where there are no submucosal glands [23], suggesting additional alternative mechanisms of BE occurrence.

## Trans differentiation of in situ squamous epithelium

Transdifferentiation is described as an irreversible metaplastic conversion from one somatic cell into another [24]. It has been confirmed that GERD mainly induced alterations of the expression of important developmental transcription factors, which causing esophageal squamous cells switch to columnar cells (transdifferentiation) or causing immature esophageal progenitor cells to undergo columnar rather than squamous differentiation (transcommitment) [25]. This theory is especially intriguing in light of the successful production of iPS cells by Yamanaka et al. [26]. They conclude that differentiated cells can be reprogrammed to an embryonic-like state having pluripotent by transfer of a few defined factors into oocytes or by fusion with embryonic stem (ES) cells.

That the differentiated cells could retro-differentiated to pluripotent cell in a particular situation is possible. The normal squamous epithelium in esophagus transforms directly into a columnar epithelial phenotype through reprogramming. The similar phenomenon has been declared during formation of gut duct when the mouse esophagus develops from the single intestine layer to squamous epithelium. According to this theory, the differentiated squamous cell needs to revert back to such an early stage that it could give rise to all three kinds of BE cell types and also be able to constantly replicate and maintain a stable population of BE cells.

Cumulative evidence showed that the squamous-to-columnar metaplasia occurs in an early intermediate stage characterized by the presence of epithelium combined squamous and columnar features, as multilayered epithelium [18,27]. In some ways, multilayered epithelium presented a similar mucin and cytokeratin profile to that of fully developed Barrett's metaplasia and also showed a large capacity for cell proliferation, differentiation, and expression of intestinal transcription factors [18]. Some prospective studies demonstrated that multilayered epithelium was strongly associated with GERD-induced inflammation of the gatroesophageal junction (GEJ) and was nearly 100% specific for BE [27,28].

## Transcommitment of esophageal squamous stem cells

Increasing interest is being generated by the hypothesis that metaplastic Barrett mucosa results from change in the differentiation of the esophageal squamous stem cells, which are induced to differentiate into a columnar epithelium with intestinal characteristics as a result of continuous exposure to injury from refluxed gastric juice [9]. Involving resident undifferentiated basal esophageal cells rather than fully differentiated squamous cells, the term transcommitment not transdifferentiation has been used. Stem cells located in the interpapillary zone of the basal cell layer of the squamous epithelium may undergo abnormal differentiation into columnar BE [29,30].

The altered basal stem cell differentiation could be because of direct toxicity of the refluxate. Bile-induced dilation of intercellular spaces allowed the refluxate to gain direct access to basal stem cells [31]. The stem cell theory is attractive, as it explains the variety of cellular phenotypes found in Barrett's esophagus, as well as how regeneration of basal stem cell is possible, and it correlates well with origin of cell intrinsic to the esophagus [32].

## Migration of embryonic cells residing (REC)

As p53 homolog, p63 maintains the "stemness" of regenerative stratified epithelia with features of proliferation and self-renewal [33,34]. p63 doesn't express in BE or EAC but presents frequently in distinctive multilayered epithelium [18], and bile salts and/or acid-induced downregulation in primary esophageal epithelium suggests that p63-mediated squamous-cell commitment may be damaged upon exposure to gastroduodenal reflux [35]. Recently, a study by Wang et al. shows that REC of the squamocolumnar junction (SCJ) are precursors of BE [36]. They found that p63 null embryos quickly initiate intestine-like metaplasia with gene expression features resembling Barrett's metaplasia driven by GERD. They trace its source to a unique embryonic epithelium that is usually impaired and substituted by p63-expressing cells. They also find that a scattered population of these embryonic cells persists in both adult mice and humans at SCJ, the source of BE. This implicates residual embryonic cell partly exists at the squamocolumnar junction of human, preferring migration to the injured esophageal mucosa by reflux-induced integrity damaging. This theory implies that BE is opportunistic a competitive result from interactions between cell lineages not from genetic alterations. Moreover, this hypothesis couldn't explain why these mouse embryonic cells failed to express CDX2, which is a very important transcription factor in human BE. This model may not duplicate with BE development in the human counterpart [23].

### Migration of bone marrow progenitors

It has been suggested that BE could originate from marrow-derived stem cells [37] and, although currently there are little data on this in the context of BE, similar situation supporting this hypothesis has been found in gastric intestinal metaplasia [38]. The hypothesis of bone marrow stem cell migration is encouraged by the observation of epithelial cells of donor origin (male) in the mucosa of the gastroesophageal junction of a female patient following bone marrow transplantation [39]. This provides proof of principle that bone marrow derived stem cells may populate the esophageal epithelium, although does not prove that it is the predominant mechanisms of clinical BE.

Although bone marrow stem cell correlates with mesoderm-derived tissues, its potential to form epithelial populations coming from ectoderm or endoderm is not clear; and whether this phenomenon simply represent a repair role of the circulating bone marrow derived cells to esophageal injury or do they specifically contribute to stem cells to generate BE needs further studies. Some studies suggested that the colonization of the acid-damaged esophagus by circulating, multipotent bone marrow stem cells are inflammatory cells which arise from the pluripotent bone marrow stem cell and not the potential progenitor cell of BE. In accordance with roles of metaplasia progenitor, however, there is not compatible incorporation pattern of bone marrow cells into BE glands.

In general, there are so many attractive hypotheses about the origin of BE, but no one finding can provide sufficient evidences to eliminate the rest of possibilities. It's hard to decide which hypothesis of BE origin is correct. Further studies will be needed to conduct to figure out this problem conclusively. It should also be remembered that more than one population of progenitor cells could be present in the human esophageal tissue, suggesting that the cellular origin of BE could be multiple [40].

## Signalling Pathways

Barrett metaplasia is considered a sequential molecular events complex provoked by GERD. The precise molecular mechanism of Barrett's metaplasia remains unknown. Several signaling pathways, including Wnt, BMP, Klf4, NFκB, Notch and sonic Hedgehog, and downstream transcription factors have been shown to play a fundamental role driving the formation of BE in the setting of GERD.

### Notch signal pathways

The Notch signaling pathway acts as a fundamental molecular signaling system that controls cell-fate decisions such as differentiation, proliferation, and apoptosis in almost all tissue types [41]. Recent findings revealed a correlationship between the Notch signalling and CDX2 expression. Induction of CDX2 in intestinal epithelium leads to expression of ATOH1 (a factor associated with Notch signaling), which effects differentiation of stem cells into goblet cells [42]. Notch signaling-induced Hes1 upregulation and ATOH1 downregulation resulted in differentiation suppression of stem cell into goblet cells.

It has been found that bile acid inhibition of Notch signaling in esophageal cells is correlated with an increase in Hath1 and CDX2 and may be one of the key processes contributing to the formation of BE [43]. Investigating effects of Notch signaling on the initiation of BE metaplasia, Vega et al. [44] found that upregulation of KLF4 partly inhibits Notch signaling in human esophageal epithelial cells, and promotes transdifferentiation of esophageal cells toward BE-like metaplasia. Tamagawa et al. [45] studied the correlationship between Notch signaling and CDX2 expression in Barrett's metaplasia, and found that bile acids-stimulated CDX2 expression induces intestinal differentiation of esophageal columnar cells by interaction with the Notch signaling pathway. The results provide a new insight to understand how esophageal epithelial transdifferentiation promotes the evolution of BE. Intensive study is necessary to clarify the role of Notch pathway in BE.

### BMP4 pathway

BMP4 is one of transforming growth factor (TGF)-family members, involved in regulating cellular differentiation, migration, and proliferation [46]. GERD associated inflammation can activate BMP pathway, while it's not active in normal squamous epithelium. A heterodimeric complex containing BMP receptor type I and type II is induced by BMPs, and the receptor complex controls downstream by phosphorylating specific BMP receptor–regulated Smads (Smad 1, 5, and 8). The P-Smad 1/5/8 comes into a heterocomplex with Smad 4, and the new complex moves into the nucleus to transcribe certain target genes, such as ID2.

Previous study indicated that the BMP pathway may play a role in the transformation of esophageal squamous cells into columnar cells [47]. Castillo et al. [48] investigated the roles of BMP4 and CDX2 in BE development, results suggesting that the columnar epithelial differentiation of BE includes BMP4 activation and early expression of CDX2. van Baal et al. [49] performed a tissue-specific miRNA profile to examine the function of miRNA-145 in the esophagus, the results imply that miRNA-145 indirectly targets BMP4 via GATA6 and is potentially involved in the development of BE.

Our previous study showed that bile salt and acid increase the BMP4 expression. Inflammatory injuries of esophageal squamous epithelium partly activated BMP pathway, as well as seen in the columnar epithelium of both human and rat tissues, and BMP-4 pathway can be interrupted effectively by Noggin, a BMP4 antagonist. Western blot assay suggested that BMP4 induces activation of smad1 and promotes protein expression of ID2 and CDX2. Our results [50] suggested that BMP4 mediates reflux-induced metaplastic transformation of inflamed esophageal squamous mucosa to columnar mucosa.

### KLF4 pathways

Krüppel-like factors (KLFs) are zinc finger containing transcription factors comprise a family of evolutionarily conserved zinc finger transcription factors that regulate numerous biological processes, including proliferation, differentiation, development and apoptosis [51]. Among them, KLF4 (gut-enriched Kruppel-like factor) effects intestinal mucosa development as a critical transcription factor, like CDX2 in some ways. Kazumori et al. [52] studied the direct effects of bile acids on KLF4 expression in cultured esophageal squamous epithelium, and revealed that KLF4 expressed strongly in Barrett's epithelium of both rat and human. Bile acids mixture increased KLF4 promoter activity of esophageal epithelium, raising mRNA and protein expression consequently. Mutation analysis of KLF4 promoter demonstrated that the binding site of NF-κB is in charge of bile acid-induced activation of the KLF4 promoter, sugestting

that bile acid-induced KLF4 expression followed by MUC2 production may play an important role in BE development.

### Hedgehog pathway

The Hedgehog signaling pathway plays an important role in embryonic development, cell proliferation, tissue polarity and carcinogenesis [53-55]. Wang et al. [56] studied the Hedgehog pathway reactivation in BE and its promoting columnar differentiation of esophageal epithelium. Results showed that epithelial Hedgehog pathways initiates BE occurrence by promoting secretion of stromal BMP4, which triggers esophageal epithelial transformation to a columnar phenotype.

### Shh signaling

Shh expression in normal esophagus remains controversial; however, aberrant Shh signaling may contribute to BE initiation and subsequent EAC progression. Shh is abnormally activated in injured esophageal epithelium exposed to bile salts and acids. Shh signaling contributed to BE development in two sequential stages. First, Shh signaling activates the target BMP4, and second, BMP4 signal activates SOX9 of epithelium [57]. Clemons et al. [58] propose that Shh-induced expression of SOX9 may represent an early molecular event in the development of esophageal columnar metaplasia. Consistent with their work, a recent study also reported evidence of Shh signaling in BE as well as EAC [59].

SOX9 is an important indirect target of Shh signalling. Wang et al. [56] identified the transcription factor SOX9 as a potential driver of BE. They showed that SOX9 is expressed in the basal epithelial cells of the developing mouse esophagus from embryonic day 12.5 up to birth but does not present in the esophageal epithelium of adult mouse. Furthermore, SOX9 is universally upregulated in BE and frequently expressed in EAC but is absent in normal human squamous esophagus. Clemons et al. [58] demonstrated that co-expressing CDX2 and SOX9 had a similar phenotype to single-expressing SOX9 of epithelium, suggesting that SOX9 independently drives columnar differentiation of squamous epithelium and expression of intestinal differentiation markers, reminiscent of BE. These results indicate that Shh-induced expression of SOX9 plays an important role in early stage of BE development.

BMP4 was also found a key regulator of Shh signalling. Shh induces BMP4 secretion of stromal fibroblasts, feeding back to the epithelium where it causes the expression of SOX9 and the induction of columnar differentiation markers [58].

### Wnt pathways

Wnt signaling effects on embryonic development at various stages and contributes to a columnar differentiation of foregut epithelium [57]. Wnt signaling could contribute to a columnar differentiation through activation of BMP signaling. It has been shown that components of Wnt signaling pathway play an important role controlling the balance between squamous and glandular differentiation in epidermal cells [60]. The key event that is activated by Wnt signals is the stabilization of b-catenin and the subsequent formation of nuclear b-catenin/Tcf complexes that can drive expression of Wnt target genes. Although abnormalities in Ecadherin and catenin signaling have been implicated predisposing BE to EAC, these pathways' roles in the primary Barrett's metaplasia have not been determined [57].

## Summary

Although BE has been known for over 50 years, the details of its pathogenesis are still unclear. Several candidate progenitor cells have been proposed, but there is as yet no consensus around any one of them. It has been reported that a number of developmental signalling pathways and transcription factors are critically important for causing mature squamous epithelium to change into columnar cells (transdifferentiation) or causing immature esophageal progenitor cells to undergo columnar rather than squamous differentiation (transcommitment). In animal research of reflux esophagitis, metaplasia develops from bone marrow stem cells that enter the blood and settle in the reflux-damaged esophagus. Studies in mouse models have suggested that metaplasia might result from upward migration of stem cells in the proximal stomach (gastric cardia) or from proximal extension of embryonic-type cells in GEJ. However, it's not well known which of these processes mainly contributes to the pathogenesis of BE in humans. Further research is required to fully understand how these pathways come into play with known environmental and host risk factors.

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