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New Mechanisms of Barrett's Esophagus Development

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Abstract

Barrett's esophagus (BE) is a pathological condition that develops as a result of metaplastic transformation of the stratified squamous non-keratinized epithelium of the mucous membrane of the distal part of esophagus into columnar epithelium of the intestinal type. The purpose of this review was to investigate novel hypotheses and mechanisms related to the development of BE, aiming to identify emerging trends and enhance understanding of the disease's pathogenesis for the purpose of preventing esophageal adenocarcinoma. A thorough investigation of recent scholarly publications was carried out to examine the mechanisms contributing to the development of BE. In the process of scrutinizing an extensive array of literature, novel pathways involving cell transdifferentiation and transcommitment were elucidated, supplementing the conventional theory of esophageal cell replacement.

Many aspects remain unclear, especially concerning the cell population from which BE originates and the molecular processes or phases involved in its progression to esophageal adenocarcinoma. These questions hold immense importance for researchers, as the answers will profoundly influence efforts in disease prevention and treatment. Although there are presently few experimental model systems accessible for the study of BE and esophageal adenocarcinoma, advancements in tissue engineering and organotypic cell culture systems utilizing human cells present promising avenues for future research into the pathogenesis and advancement of these conditions.

Key words: Barrett's esophagus, gastroesophageal reflux disease, Barrett's metaplasia, esophageal cell differentiation, esophageal dysplasia.

Introduction

BE stands as one of the foremost concerns in modern gastroenterology. This condition emerges through the metaplastic alteration of the multilayered non-keratinizing squamous epithelium of the lower esophageal mucosa into a columnar epithelium resembling that of the intestines [1]. BE typically develops in the distal part of esophagus against the background of chronic gastroesophageal reflux disease (GERD) and characterized histopathologically by the the replacement of normal squamous epithelium with intestinal-type columnar epithelium [2]. The clinical importance of BE lies in its role as a significant risk factor for the development of esophageal adenocarcinoma. Furthermore, it stands as the sole recognized precursor to esophageal adenocarcinoma, an exceptionally lethal form of cancer whose incidence has shown a concerning rise over the last fifty years [3]. In patients afflicted with BE, the metaplastic transformation occurs wherein columnar mucosa, comprising epithelial cells exhibiting characteristics of both gastric and intestinal types, replaces the esophageal squamous mucosa that has been damaged by gastroesophageal reflux disease (Figure 1) [4]. According to US guidelines, the diagnosis of Barrett's disease requires its endoscopic confirmation with the presence of columnar mucosa extending at least 1 cm proximal to the esophago-gastric junction, and histological evidence of intestinal-type metaplasia [4].

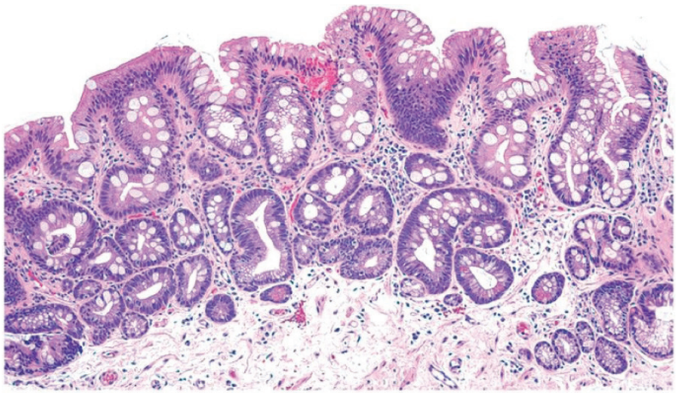

Figure 1 - Barrett's intestinal metaplasia with mucin-secreting gastric foveolar-type cells and prominent intestinal-type goblet cells.

(Photomicrograph provided by Robert Genta).

Materials and methods

We undertook a systematic search of pertinent medical databases spanning the last 15 years to gather relevant literature for our study. In accordance with the Cochrane collaboration recommendations, we conducted searches in Medline, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL). Additionally, we explored relevant publications in databases such as Web of Science and Scopus, as well as in the "Russian Medicine" database based on e-library.ru. The search terms used for literature search included "Barrett's esophagus", "gastroesophageal reflux disease", "Barrett's metaplasia", "esophageal cell differentiation", and "esophageal dysplasia".

We established specific inclusion and exclusion criteria for publications to ensure the thoroughness and accuracy of our review process. Thus, primary attention was directed towards multicenter randomized clinical trials, cohort studies with large sample sizes, and with proper statistical analysis. The review analysis did not include descriptions of individual clinical cases or case series. The authors analyzed each article, paying attention to the quality of each publication, study design, sample size, quality of statistical analysis of results, and completeness of reference citations. An essential criterion for the inclusion of a publication in the review was the presence in the study protocol of mandatory histological examination of patients with BE.

Discussion

The established mechanism previously known for the development of BE is associated with an increase in the intensity and extent of esophageal damage caused by acid, bile, and pancreatic enzyme reflux [2, 4]. Activation of cyclooxygenase-2 (COX-2) occurs under the influence of bile salts, and experiments with laboratory rats have shown that inhibiting its activity results in a decreased risk of cancer development. Patients diagnosed with dysplasia and cancer frequently exhibit increased levels of COX-2 suppression. In vitro research has revealed that intermittent (pulse-like) acid exposure to the esophageal mucosa exerts a more pronounced effect on epithelial proliferation compared to continuous exposure.

Nevertheless, the pathogenetic mechanism underlying the development of metaplasia in BE remains unclear. It is proposed that the appearance of metaplasia arises from continual exposure to aggressive substances that damage mature cells, simultaneously stimulating the distorted differentiation of immature proliferating cells (gastric acid, bile acids, and pancreatic enzymes), which damage mature esophageal epithelial cells [1]. Indeed, at a certain stage, intestinal metaplasia appears to be an adaptive response that promotes the formation of columnar epithelium, which is more resistant to damage from various pathological factors. The reflux of bile acids and pancreatic enzymes causing damage to the esophageal mucosa results in the onset of "chemical" esophagitis in the terminal part of the esophagus. This condition is distinguished by dystrophic and inflammatory alterations in the mucous membrane, which may include the appearance of intestinal metaplasia [2, 5].

The article authored by researchers from North Carolina examine two histological variants of esophageal cancer: squamous cell carcinoma and adenocarcinoma, and explore the contribution of BE to their pathogenesis. They also underscore the importance of investigating the pathogenesis of BE and adenocarcinoma to enable effective risk stratification and facilitate the development of treatments [2].

There is a suggestion that BE may originate from either differentiated cells or stem cells. Within this framework, in the article investigates four potential cellular sources that may contribute to the development of BE. At the molecular level, metaplasia is presumably caused by activation or inactivation of transcription factors. The article conducts an analysis of microchip and SAGE data to identify potential "drivers" and "passengers" involved in the development of BE [6].

Additionally, potential cellular origins for BE are contemplated, encompassing basal cells of the squamous epithelium, submucosal gland cells of the esophagus, cells originating from the upper part of the stomach, and specialized cells located at the esophagogastric junction (Figure2) [1].

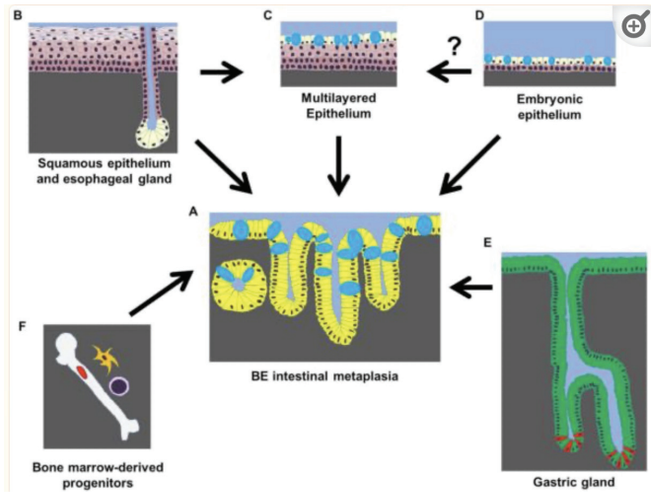

Figure 2 - Potential Cellular Substrates for Barrett's Esophagus (BE).

Columnar epithelial cells with glandular cells (mucin depicted as blue oval shapes) in (A). Direct sources of BE may be flat epithelial cells of the esophageal epithelium (keratinocytes) or ductal epithelial cells of the esophageal submucosal glands (B). Mucosal eosinophilic change (MLE) is suspected to be a precursor of BE (C). MLE is associated with esophageal glands in humans. BE may be caused by migration of residual embryonic esophageal cells or reactivation of developmental pathways (e.g., BMP4, Hh) (D). Possible variants include migration of gastric cells (e.g., Lgr5- positive stem cells in red at the base of glands) (E) and circulating bone marrow precursors (F).

1. Transcription factors: P63, Sox2, and Pax9

P63 acts as a critical regulator of epithelial stratification and progenitor cell survival in esophageal squamous epithelium. Sox2 is identified as an oncogene in lung cancer and esophageal squamous cell cancer [7, 8]. Pax9 is involved in the regular process of differentiating esophageal squamous epithelium [9].

2. Intestinal transcription factors: Cdx1 and Cdx2, HNFs, GATA4, and GATA6

Cdx1 and Cdx2 are pivotal regulators of intestinal development. HNFs are involved in gene regulation within the liver, pancreas, and intestine. GATA4 and GATA6 contribute to the differentiation of mesodermal and endodermal tissues [10, 11, 12].

3. Signaling pathways: TGFβ/BMP, WNT, NFkB, Hedgehog, Notch

The significance of BMP4 in inducing metaplasia in GERD is highlighted, alongside NFkB activation in gastroesophageal reflux [13, 14]. The influence of WNT on Cdx1 and Cdx2, as well as its regulation of Sox9, is acknowledged [15]. Hedgehog activation and Notch inhibition are also considered potential factors in BE development [16].

4. Stromal factors

The interaction between epithelial-mesenchymal cells plays a pivotal role in epithelial cell differentiation. The association between inflammation and BE, driven by proinflammatory cytokines, is well-studied. Inflammatory alterations occur before cell damage in GERD, with esophageal squamous epithelial cells secreting chemokines such as IL8 and IL1β, initiating spontaneous inflammation and metaplasia. Metaplasia, characterized by TFF2 and Cdx2 expression, is stimulated by bile acid exposure [17-19].

5. MicroRNAs

Several studies have demonstrated alterations in microRNA profiles in individuals with BE and adenocarcinoma. Some microRNAs (e.g., miR-203) express key genes associated with BE (e.g., p63), while others modulate the expression of such genes. Transcription factors like p63 are known to modulate microRNA processing, such as miR-21. The role of microRNAs as drivers or passive participants in BE development is yet to be determined [20-22].

6. Other factors

The level of retinoic acid increases in BE tissues compared to normal esophagus and decreases with adenocarcinoma. Changes in retinoic acid receptors are observed, and treatment with choleretic drugs enhances the activity of retinoic acid receptors, further promoting the differentiation of esophageal epithelial cells into columnar cells.

The RUNX3 gene, part of the transcription factor family with a regulatory RUNT domain, is crucial for esophageal cellular differentiation. Loss of RUNX3 results in gastric epithelium differentiation into intestinal-type cells, which may contribute to BE development [23-26].

7. Transcommitment

Transcommitment, a process akin to transdifferentiation, shares similarities with paligenosis in that it involves the initial dedifferentiation of mature cells into progenitor-like cells, followed by abnormal redifferentiation [27]. However, unlike transdifferentiation, transcommitment begins with immature progenitor cells that undergo abnormal differentiation, potentially triggered by factors such as GERD. This process could elucidate why different cell types persist even when GERD is managed [16, 27].

The exact progenitor cells responsible for Barrett's metaplasia are not fully elucidated, but four categories of candidates are proposed (Figure 3) [28]: progenitor cells native to the esophagus, those from the proximal stomach (gastric cardia), specialized populations at the esophago-gastric junction (EGJ), and bone marrow progenitor cells transported to the esophagus.

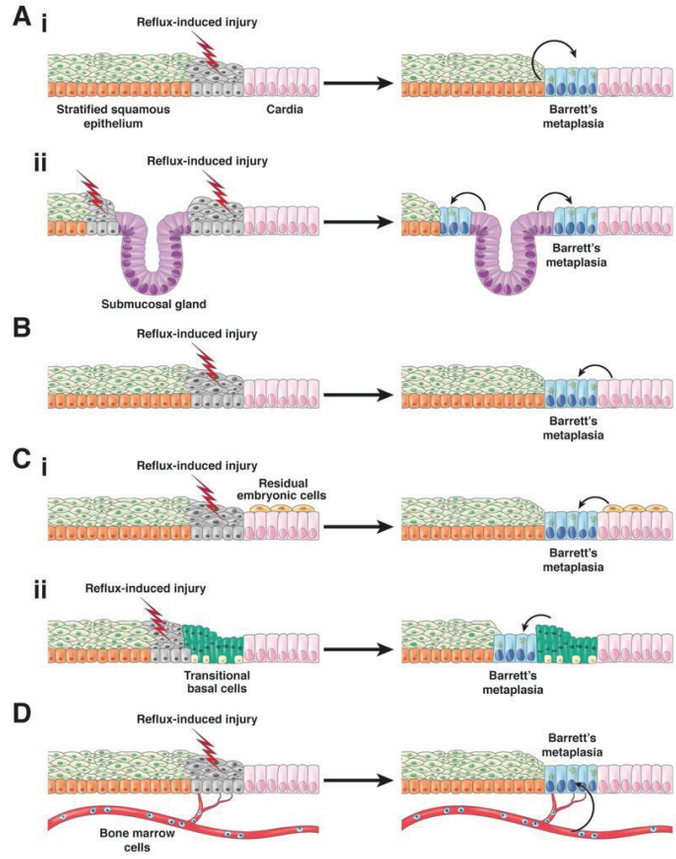

Figure 3 - Proposed cells of origin for BE.

1) Cells native to the esophagus including (1a) squamous epithelial cells that undergo reflux-induced transdifferentiation or transcommitment to produce the columnar cells of Barrett's metaplasia and (1b) Progenitor cells in esophageal submucosal glands and/or their ducts. 2) Progenitor cells in the gastric cardia. 3) Specialized populations of cells at the esophago-gastric junction migrate into the reflux-damaged esophagus including (3a) residual embryonic cells (RECs) or (3b) transitional basal cells (TBCs). 4) Circulating bone marrow cells. For all of these proposed progenitor cells, reflux-induced injury to the esophageal squamous mucosa is assumed to initiate the metaplastic process, perhaps by stimulating progenitor cell migration into the damaged esophagus via a wound-healing process. In addition, reflux is assumed to induce the transcommitment of the progenitor cells to produce the multiple columnar cell types of Barrett's metaplasia. (Figure modified from Jiang et al [14]).

Furthermore, research by Jiang et al. suggests that transitional epithelium is inherent and instigates metaplasia. They observed distinctive expression patterns of three protein markers—cytokeratins KRT5 and KRT7, and the transcription regulator p63—in cell types at the murine gastroesophageal junction [1]. KRT7 expression in BE cells in humans is particularly characteristic of transitional epithelium [29].

Jiang and colleagues discovered a group of cells, different from RECs, located at the transitional zone of the SCJ in mice and humans. These cells are believed to potentially lead to BE [30]. Their research revealed that this transitional epithelium is sustained by a group of precursor cells referred to as transitional basal cells (TBCs). The TBCs expressed squamous markers such as KRT5, KRT14, and p63, along with a columnar cytokeratin characteristic of Barrett's metaplasia (KRT7+), which differed from nearby squamous basal cells and cardia mucosal progenitor cells (Figure 4) [30]. Following the establishment of an esophago-jejunostomy to stimulate bile reflux, Jiang and team observed an increase in proliferation and differentiation of TBCs into columnar epithelium expressing genes typical of intestinal cells, such as Cdx2. Additionally, the overexpression of Cdx2 in TBCs facilitated their differentiation into intestinal-type epithelial cells [1].

Additionally, a mechanism contributing to the onset of BE is chronic reflux esophagitis, triggered by gastroesophageal reflux and other harmful substances, including exposure to high concentrations of nitric oxide (NO) derived from dietary nitrates found in green leafy vegetables. The majority of ingested nitrate is absorbed in the small intestine and excreted the body unchanged through urine.

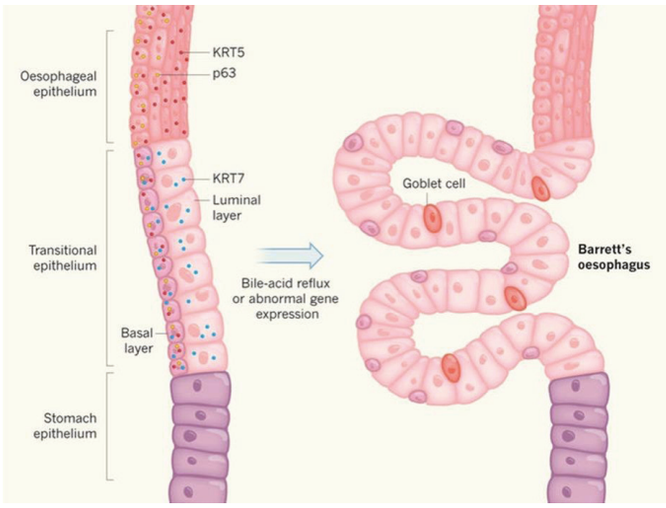

Figure 4 - Cross-section of epithelial cell types in the esophagus.

The gastroesophageal junction in mammals consists of various epithelial cells. Jiang et al. describe the transitional epithelial zone between the esophagus and the stomach, showing differential expression of three marker proteins (KRT5, KRT7, and p63) in the basal and luminal layers, and surrounding epithelium. They provide evidence in mice and humans that bile reflux or abnormal gene expression can cause abnormal expansion of this transitional epithelium, forming precancerous tissue containing goblet cells.

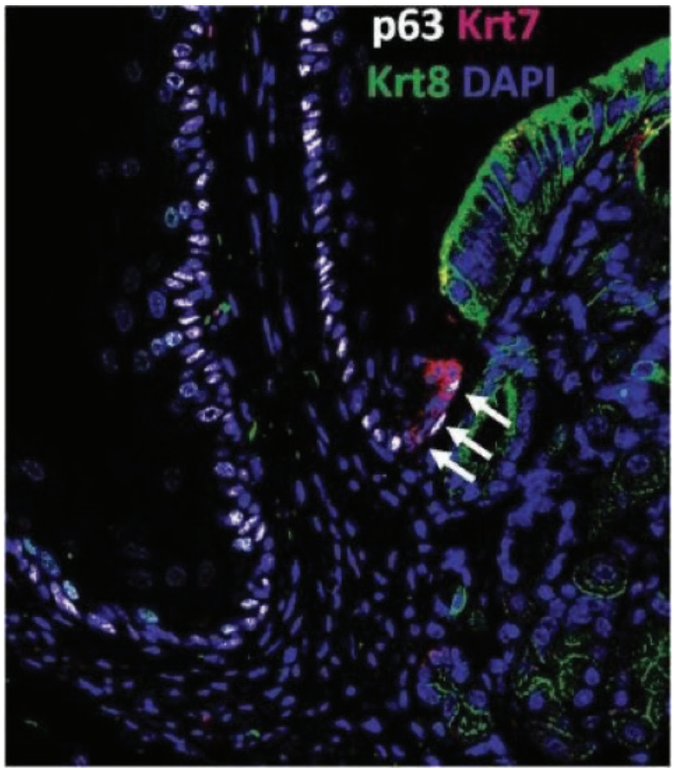

Figure 5 - Transitional basal cells at the mouse squamo-columnar junction.

Ttransitional basal cells (p63+ Krt7+ Krt8-negative) located at the squamo-columnar junction. The neighboring squamous cells (p63+, Krt7- negative, Krt8-negative) on the left and the columnar gastric cells (p63-negative, Krt7-negative, Krt8+) on the right (see details in Jiang et al[14]).

However, around 25% of it is concentrated by salivary glands and secreted into the mouth, where bacteria on the tongue convert recycled nitrate into nitrite. After being swallowed, nitrite comes into contact with acidic gastric juice and quickly transforms into nitric oxide (NO). Increased levels of NO have been observed at the gastroesophageal junction following the consumption of nitrates [31, 32].

Scientists from Tohoku University Graduate School of Medicine (Japan) investigated the proposition that elevated NO levels affect the Rho/ROCK signaling pathway in esophageal fibroblasts, potentially leading to aberrant wound healing characterized by delayed wound contraction. This phenomenon, they hypothesized, could contribute to the onset of BE [31, 33].

The study provides an overview of the molecular, immunological, and genetic mechanisms involved in BE development.

In 1950, British surgeon Norman Barrett coined the term "Barrett's esophagus" and described the classical mechanism of its development, characterized by changes in the epithelium of the lower esophagus, which can occur as a result of chronic acid reflux from the stomach. Initially, there is incompetence of the gastroesophageal barrier function, leading to the development of reflux containing acid, bile, and pancreatic enzymes. Under the influence of aggressive factors from gastric juice, the stratified squamous epithelium of the esophagus is destroyed, and it is replaced by columnar and intestinal epithelium [2].

In 2009, Spechler SJ and Rhonda F. Souza proposed an alternative concept of reflux esophagitis development, suggesting that the pathology begins with cytokine-mediated injury rather than acidic chemical exposure [1, 34].

Jiang M. and colleagues from the UK provided evidence supporting the idea that transitional epithelium is innate and initiates metaplasia. The authors identified differences in the expression of cytokeratins KRT5 and KRT7, as well as the transcription regulator p63, delineating cell types in the murine gastroesophageal junction. It was confirmed that KRT7, expressed in BE cells in humans, is specific to transitional epithelium [30].

Researchers from North Carolina identify potential cellular sources such as differentiated and stem cells. Additionally, the role of transcription factors, including P63, Sox2, Pax9, as well as intestinal factors and signaling pathways (TGFβ/BMP, WNT, NFκB, Hedgehog, Notch), is discussed in the context of BE development [7-9].

Stromal factors, epithelial-mesenchymal cell interactions, and inflammation, particularly the association with proinflammatory cytokines, are considered key in BE development. MicroRNAs, such as miR-203, are also extensively analyzed in light of their role in regulating genes associated with BE [17-19].

Factors such as retinoic acid levels, RUNX3, KLF4, and KLF5 genes are highlighted in the context of esophageal epithelial cell differentiation [26]. The study provides important data for understanding the molecular mechanisms of esophageal pathologies development, which may contribute to effective risk stratification and treatment methods development [23- 25].

Results

After analyzing the latest data on this topic, we have reached the following conclusions:

The research carried out by scientists from North Carolina emphasized the importance of recognizing two histological types of esophageal cancer – squamous cell carcinoma and

adenocarcinoma – and explored the role of precision medicine in their classification and therapy.

Transcription factors such as P63, Sox2, and Pax9 play a key role in the development of esophageal squamous epithelium. Interference in their activity provides promising targets for therapeutic interventions. This also emphasizes the need for detailed molecular-level analysis to understand metaplasia and the activation/inactivation of transcription factors.

Signaling pathways such as TGFβ/BMP, WNT, NFκB, Hedgehog, and Notch represent a complex network of interconnections that influence the development of BE. Identifying BMP4 as a key player in the induction of metaplasia in gastroesophageal reflux reveals potential points of intervention for preventing this process.

Stromal factors and their interaction with epithelial-mesenchymal cells emphasize the role of inflammation in the development of BE. This is associated with pro-inflammatory cytokines such as IL8 and IL1β, highlighting the importance of comprehensive study not only of epithelial but also of mesenchymal aspects.

MicroRNA profiles are an additional aspect of the molecular heterogeneity of BE and adenocarcinoma. Studying the impact of microRNAs such as miR-203 on key genes related to BE provides new opportunities for understanding and therapeutically affecting the developmental processes of these dangerous conditions.

Changes in the levels of retinoic acid in esophageal tissues under various conditions raise questions about its impact on cellular differentiation. Discussions of molecular mechanisms associated with retinoic acid receptors can complement the understanding of the connection between these changes and the development of pathology.

The role of the RUNX3 gene in esophageal cellular differentiation and its loss in the context of Barrett's development are subject to discussion. Analyzing the mechanisms by which the loss of RUNX3 affects cell types may reveal pathogenetic processes.

The consideration of KLF4 and KLF5 involvement in the differentiation of squamous epithelium in the esophagus highlights their possible role in the formation of BE.

The use of immortalized cell lines was discussed, emphasizing the importance of model systems for studying the molecular mechanisms of Barrett's development. The limitations of current methods highlight the need for new approaches for more accurate modeling of the precancerous state.

The possible link between chronic reflux and high levels of nitric oxide was also discussed. This discussion may shed light on the impact of environmental factors on the development of the disease".

Conclusion

Much remains unknown, particularly: from which cellular population the cells originate and what are the molecular events or stages through which BE progresses to esophageal adenocarcinoma. These are crucial questions for researchers, the answers to which will significantly impact disease prevention and treatment. Despite the current lack of extensive experimental model systems for studying BE and esophageal adenocarcinoma, advancements in tissue engineering and organotypic cell-based culture systems provide promising avenues for future research into the pathogenesis and progression of these conditions.

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References

1. Que J, Garman KS, Souza RF, Spechler SJ.Pathogenesis and Cells of Origin of Barrett's Esophagus. *Gastroenterology*. 2019; 157(2): 349–364. https://doi.org/10.1053/j.gastro.2019.03.072.
2. Chen H, Fang Y, Tevebaugh W, Orlando RC, Shaheen NJ and Chen X. Mechanism of Barrett's Esophagus. *Dig Dis Sci.* 2011; 56(12): 3405–3420. https://doi.org/10.1007/s10620-011-1885-6.
3. Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, Smith CM, Michael MZ. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. *Br J Surg.* 2010; 97: 853–61. https://doi.org/10.1002/bjs.7000.
4. Spehler SY, Sousa RF. Esophagus Barretta. N Engl J Med. 2014; 371: 836-45. https://doi.org/10.1056/NEJMra1314704.
5. Shaheen NJ, Falk GW, Iyer PG, Gerson LB; American College of Gastroenterology. ACG Clinical Guideline: Diagnosis and Management of Barrett's Esophagus. Am J Gastroenterol 2016; 111:30–50. https://doi.org/10.1038/ajg.2015.322.
6. Wang J, Qin R, Ma Y, et al. Differential gene expression in normal esophagus and Barrett's esophagus. *J Gastroenterol.* 2009; 44: 897–911. https://doi.org/10.1007/s00535-009-0082-2.
7. Bass AJ, Watanabe H, Mermel CH, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat Genet.* 2009; 41: 1238–42. https://doi.org/10.1038/ng.465.
8. Mutoh H, Sashikawa M, Sugano K. Sox2 expression is maintained while gastric phenotype is completely lost in Cdx2-induced intestinal metaplastic mucosa. *Differentiation*. 2010; 81: 92–8. https://doi.org/10.1016/j.diff.2010.10.002.
9. Sherwood RI, Chen TY, Melton DA. Transcriptional dynamics of endodermal organ forquanmation. *Dev Dyn*. 2009; 238: 29–42. https://doi.org/10.1002/dvdy.21810.
10. Benoit YD, Pare F, Francoeur C, et al. Cooperation between HNF-1alpha, Cdx2, and GATA-4 in initiating an enterocytic differentiation program in a normal human intestinal epithelial progenitor cell line. *Am J Physiol Gastrointest Liver Physiol.* 2010; 298: G504–17. https://doi.org/10.1152/ajpgi.00265.2009.

11. Kazumori H, Ishihara S, Kinoshita Y. Roles of caudal-related homeobox gene Cdx1 in oesophageal epithelial cells in Barrett's epithelium development. *Gut*. 2009; 58: 620–8. https://doi.org/10.1136/gut.2008.152975.
12. Verzi MP, Shin H, Ho LL, Liu XS, Shivdasani RA. Essential and redundant functions of Caudal family proteins in activating adult intestinal genes. *Mol Cell Biol.* 2011: 31(10): 2026–2039. https://doi.org/10.1128/MCB.01250-10.
13. Domyan ET, Ferretti E, Throckmorton K, Mishina Y, Nicolis SK, Sun X. Signaling through BMP receptors promotes respiratory identity in the foregut via repression of Sox2. *Development.* 2011; 138: 971–81. https://doi.org/10.1242/dev.053694.
14. Rodriguez P, Da Silva S, Oxburgh L, Wang F, Hogan BL, Que J. BMP signaling in the development of the mouse esophagus and forestomach. *Development*. 2010; 137: 4171–6. https://doi.org/10.1242/dev.056077.
15. Yamanaka Y, Shiotani A, Fujimura Y, et al. Expression of Sonic hedgehog (SHH) and CDX2 in the columnar epithelium of the lower oesophagus. *Dig Liver Dis.* 2011; 43 :54–9. https://doi.org/10.1016/j.dld.2010.04.014.
16. Wang DH, Clemons NJ, Miyashita T, et al. Aberrant epithelial-mesenchymal Hedgehog signaling characterizes Barrett's metaplasia. *Gastroenterology*. 2010; 138: 1810–22. https://doi.org/10.1053/j.gastro.2010.01.048.
17. Lao-Sirieix P, Fitzgerald RC. Role of the micro-environment in Barrett's carcinogenesis. *Biochem Soc Trans.* 2010; 38: 327–30. https://doi.org/10.1371/journal.pone.0000177.
18. Menke V, van Es JH, de Lau W, et al. Conversion of metaplastic Barrett's epithelium into post-mitotic goblet cells by gamma-secretase inhibition. *Dis Model Mech.* 2010; 3: 104–10. https://doi.org/10.1242/dmm.003012.
19. Quante M, Abrams JA, Marrache F, Wang TC. Barrett's esophagus correlates with increased putative gastrointestinal stem cell markers DCLK1 and CCK2R in a IL1b mouse model and in humans. *Gastroenterology*. 2010; 138 (5): S-128-S-128. https://doi.org/10.1016/S0016-5085(10)60586-0.
20. Bansal A, Lee IH, Hong X, et al. Feasibility of MicroRNAs as Biomarkers for Barrett's Esophagus Progression: A Pilot Cross-Sectional, Phase 2 Biomarker Study. *Am J Gastroenterol.* 2011; 106(6): 1055-1063. https://doi.org/10.1038/ajg.2011.37.
21. Fassan M, Volinia S, Palatini J, et al. MicroRNA expression profiling in human Barrett's carcinogenesis. *Int J Cancer.* 2010. https://doi.org/10.1002/ijc.25823.
22. Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, Smith CM, Michael MZ. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. *Br J Surg.* 2010; 97: 853–61. https://doi.org/10.1002/bjs.7000.
23. Gudas LJ, Wagner JA. Retinoids regulate stem cell differentiation. J Cell Physiol. 2011; 226: 322–30. https://doi.org/10.1002/jcp.22417.
24. Hu D, Wan Y. Regulation of Kruppel-like factor 4 by the anaphase promoting complex pathway is involved in TGF-beta signaling. *J Biol Chem.* 2011; 286: 6890–901. https://doi.org/10.1074/jbc.M110.179952.
25. Kazumori H, Ishihara S, Takahashi Y, Amano Y, Kinoshita Y. Roles of Kruppel-like factor 4 in oesophageal epithelial cells in Barrett's epithelium development. *Gut*. 2011; 60: 608–17. https://doi.org/10.1136/gut.2008.152975.
26. Zheng H, Pritchard DM, Yang X et al. KLF4 gene expression is inhibited by the notch signaling pathway that controls goblet cell differentiation in mouse gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol.* 2009; 296: G490–8. https://doi.org/10.1152/ajpgi.90393.2008.
27. Van DH, Sousa RF. Transcription: the way to Barrett's metaplasia. *Adv Exp Med Biol*. 2016; 908: 183-212. https://doi.org/10.1007/978-3-319-41388-4_10.
28. Jin RU, Mills JC. Are gastric and esophageal metaplasia relatives,the case for Barrett's stemming from SPEM. *Dig Dis Sci.* 2018; 63: 2028–2041. https://doi.org/10.1007/s10620-018-5150-0.
29. Lizhe Zhuang & Rebecca C. Fitzgerald Origins in the oesophagus. Nature. 2017; 550: 463–464. https://doi.org/10.1038/nature24150.
30. Jiang M, Li H, Zhang Y et al. Transitional basal cells at the squamous-columnar junction generate Barrett's oesophagus. *Nature*. 2017; 550: 529–533. https://doi.org/10.1038/nature24269.
31. Asanuma K, Huo X, Agoston A, Zhang X, Yu C, Cheng E, Zhang Q, Dunbar KB, Pham TH, Wang DH, Iijima K, Shimosegawa T, Odze RD, Spechler SJ, Souza RF: In oesophageal squamous cells, nitric oxide causes s-nitrosylation of akt and blocks Sox2 (sex determining region y-box 2) expression. *Gut* 2015. https://doi.org/10.1136/gutjnl-2015-309272.
32. Kusaka G, Uno K, Iijima K, Shimosegawa T. Role of nitric oxide in the pathogenesis of Barrett's-associated carcinogenesis. *World J Gastrointest Pathophysiol.* 2016 Feb 15; 7(1): 131–137. https://doi.org/10.4291/wjgp.v7.i1.131.
33. Fujiya T, Asanuma K, Koike T, Okata T, Saito M, Asano N, Imatani A, Masamune A. Nitric oxide could promote development of Barrett's esophagus by S-nitrosylation-induced inhibition of Rho-ROCK signaling in esophageal fibroblasts. *Am J Physiol Gastrointest Liver Physiol.* 2022; 322 (1): G107-G116. https://doi.org/10.1152/ajpgi.00124.2021.
34. Wang DH. Souza RF. Transcommitment: paving the way to Barrett's metaplasia. *Adv Exp Med Biol.* 2016; 908: 183-21. https://doi.org/10.1007/s10620-018-5069-5.