

MECHANISTIC APPROACH OF ESOPHAGEAL CANCER DUE TO MICROBIAL GROWTH

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ABSTRACT

One of the main subtypes of esophageal cancer (EC), esophageal adenocarcinoma (EAC) is a developing worldwide health issue. The incidence of EAC has been continuously increasing in spite of gains in survival rates. Although genetic factors to EAC have been extensively studied, little is known about the involvement of the human microbiome in its pathophysiology. Recent research indicates a link between changes in the microbial ecology and the advancement of EAC, with particular bacterial genera acting as possible early detection indicators. Gastroesophageal reflux disease (GERD) is a significant risk factor for EAC because it causes long-term inflammation and molecular alterations that result in Barrett's esophagus, a condition that precedes EAC. Long-term exposure to acid and bile causes Barrett's esophagus, which in turn causes cellular change via pathways involving DNA damage, oxidative stress, and resistance to apoptosis. Important genetic changes, including modifications to COX-2, p53, and cell cycle regulators, are linked to the transition from Barrett's esophagus to EAC. Depending on the stage of the disease, endoscopic resection, chemotherapy, radiation therapy, targeted therapy, and immunotherapy are among

the current treatment options for EAC. Although surgical procedures and proton pump inhibitors (PPIs) are used to treat GERD symptoms, their ability to stop the spread of malignancy is still up for discussion. Furthermore, innovative approaches to managing EAC risk include dietary changes, biomarkers for early identification, and developing treatment techniques including microbiota modification. Clarifying the molecular processes behind GERD-induced carcinogenesis, improving biomarker-based diagnostic methods, and creating treatments that target the microbiota should be the main goals of future research. Improving early detection, prevention, and individualized treatment plans requires an understanding of the complex interactions of genetic, microbial, and environmental variables in the course of EAC.

Keywords: *Barrett's Esophagus, Bile Reflux, Carcinogenesis, Dysplasia, Gastroesophageal Reflux Disease (GERD), Human Microbiome, Metaplasia, Oxidative Stress*

Introduction

Esophageal adenocarcinoma (EAC) is one of the main subtypes of esophageal cancer. While 5-year relative survival rates were low throughout the previous 20 years, the incidence rate of EAC gradually climbed. More research has been done on the mechanism of EAC in connection to genetic variables than in relation to the human microbiome. Studies on the connection between EAC and the human microbiota are now in their infancy (Dan et al.,2022).The seventh most frequent malignancy, esophageal cancer (EC), is expected to cause 604,000 new cases globally in 2020. Additionally, it ranks as the sixth most common cause of cancer-related mortality, with an anticipated 544,000 deaths in 2020(Sung et al.,2021). Except in Korea and Japan, the age-standardized 5-year net survival of EC patients ranged from 10 to 30 percent between 2010 and 2014. Between 2000 and 2014, the age-standardized 5-year net survival trends in several nations rose by 6–10% (Allemani et al.,2018).Although esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two main subtypes of EC, there are notable differences between the two in terms of risk factors, regional patterns, and temporal trends (Abnet et al.,2018; Uhlenhopp et al.,2020).

One of the biggest concerns of this century is addressing the EC's worldwide load. The genetic composition of humans is almost the same. The human microbiome's metagenome exhibits more variety than the human genome (Lloyd-Price et al., 2016). The human microbiota is a very unique, intricate, and ever-changing community found in every healthy person (Consortium, 2012; Gilbert et al., 2018). The human body's largest microbial habitat, the digestive tract is home to the greatest variety and quantity of microorganisms (Gupta et al., 2017; Adlerberth & Wold, 2009). There is a correlation between EC symptoms and the course of the illness. Patients may present with progressive dysphagia, persistent back or retrosternal pain, and noticeable cachexia in the latter stages, but in the early stages, they may be asymptomatic or complain of dysphagia. In early-stage disease, endoscopic treatment is frequently utilized to remove malignant material. For locally advanced disease, chemotherapy and radiation therapy are typically administered either prior to or following surgical resection. It has been determined that obesity and gastroesophageal reflux disease (GERD) are significant risk factors for EAC. Alcohol use and tobacco use may promote the development of EAC (Coleman et al., 2018;). For metastatic disease, treatment options include chemotherapy, radiation therapy, immunotherapy, targeted therapy, or a combination of these treatments to control symptoms and slow the progression of the disease (Rubenstein & Shaheen, 2015; Li et al., 2023; Dan et al., 2022). The three primary community types that make up the esophageal microbiota have been shown to differ significantly from one another. *Streptococcus* in type 2 and *Prevotella* in type 3 are the two most common genera among them. In addition to *Streptococcus* and *Prevotella*, type 1 is an intermediate between types 1 and 2, containing higher concentrations of *Haemophilus* and *Rothia* (Deshpande et al., 2018; Kaakoush et al., 2015). According to recent research, esophageal carcinogenesis is associated with changed human microbial communities and decreased microbial diversity, particularly among *Enterobacteriaceae*, *Campylobacters*, acid-producing bacteria, and periodontal pathogens (*Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*). *Tannerella forsythia*, *Helicobacter pylori*,

Prevotella, and Aggregatibacter actinomycetemcomitans can all be used as biomarkers for EAC screening and individualized precision diagnosis. The classification of the oral microbiota, the expression of biomarkers linked to HPV, and the DNA status of Fusobacterium nucleatum can all be significant prognostic indicators of EAC. Notably, new clinical treatments pertaining to the human microbiota, including as proper oral hygiene, a diet high in fiber and low in fat, exercise, PPI, mucosal protective drugs, and probiotics, may also be used to treat EC. These treatments may be very beneficial to patients (Chen et al.,2017).

Gastroesophageal reflux disease (GERD)

Esophageal cancer and gastroesophageal reflux disease (GERD) are strongly linked. Given that the target organs for GERD lack the esophageal's inherent protective mechanisms, the carcinogenic qualities of the gastroduodenal contents may also cause cancer in other organs .With an increasing incidence, gastroesophageal reflux disease (GERD) is a highly common condition. The illness is unquestionably connected to the pathophysiology of Barrett's esophageal adenocarcinoma. Given that the target organ for GERD lacks the esophageal inheritance protective mechanisms, the carcinogenic qualities of the gastroduodenal contents may also cause cancer in other organs (Herbella et al.,2015).

GERD-induced carcinogenesis mechanism:

The most researched cancer associated with GERD is esophageal adenocarcinoma, which started in Barrett's esophagus. Nevertheless, little is known about its cellular causes and molecular mechanisms (Fang et al.,2013).

Esophageal inflammation brought on by GERD causes oxidative stress, which damages DNA. bile and acid both have an effect on carcinogenic pathways. Acid causes apoptosis, reduces proliferation, and damages DNA. Bile salts produce resistance to apoptosis,damages DNA, and has a pH-dependent effect on proliferation (Fang et al.,2013;Denlinger & Thompson,2012).

Barrett's esophagus

Barrett's esophagus is intimately associated with long-term exposure to acid columnar epithelium. Theoretically, squamous epithelium could metaplasia and develop into intestinalized columnar epithelium via two separate route methods. In the first pathway, mature squamous cells undergo direct transdifferentiation into intestinal cells. On the other hand, esophageal intestinal mucosal cells might be the consequence of aberrant stem cell differentiation in the esophageal mucosa (Dvorak et al.,2011).

Male sex, advanced age, cigarette smoking, obesity with an intra-abdominal distribution of body fat, hiatal hernia, chronic GERD, and white race are risk factors for Barrett esophageal cancer. Approximately 0.25% of patients without dysplasia and 6% of patients with high-grade dysplasia are at risk for esophageal cancer each year. According to high-quality research, persons with Barrett esophagus who receive medication or surgical treatment for their GERD do not significantly differ in their risk of developing cancer. Patients with high-grade dysplasia who receive endoscopic eradication therapy with radiofrequency ablation had a much lower chance of developing cancer(Spechler, 2013).

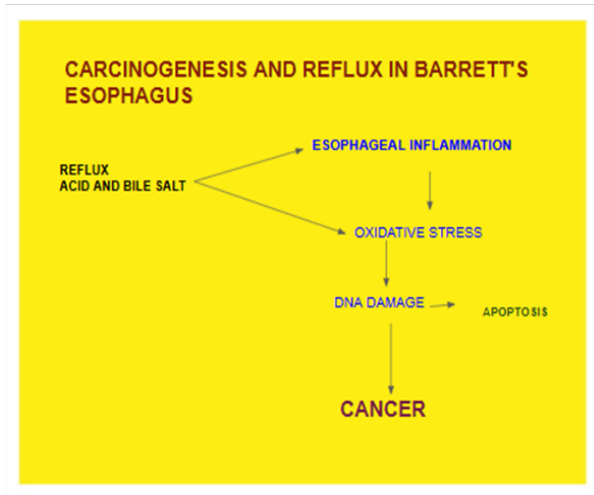
How GERD may induce Barrett's metaplasia

GERD that persists over time might lead to Barrett's metaplasia. By triggering transcription factors or generating developmental signaling pathways that establish an intestinal phenotype, elements of the refluxed gastric juice (such as acid or bile salts) and/or the ensuring esophageal inflammation (reflux esophagitis) may result in esophageal metaplasia (Wang & Souza, 2011).

Progression of Barrett's esophagus to esophageal cancer:

The risk of developing esophageal cancer is 30-150 times higher for patients with Barrett esophagus than for those without Barrett. Patients with Barrett esophagus have a 0.5% to 1.0% annual chance of cancer advancement. The molecular mechanisms underlying the metaplastic cells malignant transformation are still being understood. The idea that gastric

and bile reflux have a causal role has been validated by in vitro studies. The histologic alteration of the distal esophageal mucosa that lead to cancer when repeatedly exposed to bile salts in an acidic environment over a protracted period of time are replicated by immortalized non transformed esophageal Barrett cells cultivated in cell culture (Das et al.,2011;Denlinger & Thompson ,2012).When cells were treated for five minutes every day to glycochenodeoxycholic acid at pH of 4 . The expression of the colon-specific antigen Das-1 revealed phenotypic alteration as early as two weeks. Following 30 weeks of daily treatment, the histologic alteration showed that the cells had rounded and started to form units that resemble glands. More aggressive growth patterns were observed over 65 weeks later, and in a naked animal model, the cells developed tumorigenic properties. Numerous genes were found to become activated through analysis of the molecular processing that takes place concurrently with the morphologic alterations. Between weeks 22 and 62, the most noticeable alteration was 10-to 20-fold rise in COX-2 expression. In addition, there was a 2-fold rise in p53 depression and a 3--fold increase in TC22 expression. Furthermore, as cells evolved into a more aggressive phenotype, many factors involved in cell cycle regulation were elevated. After 20 weeks of daily bile acid exposure, ATM (ataxia telangiectasia , mutated) and ATR (ATM and Rad3-related), crucial cell cycle checkpoint regulator that stop cells from leaving values at 20 weeks showed that the following genes were elevated in comparison to untreated control cells: RB1 (2.18) , E2F1 (5.55), CCNE1 (6.56), CDC25A (8.24), BRCA1 (3.82), TP53 (2.2) and CHEK2 (2.13) (Fang et al.,2011;Denlinger & Thompson ,2012). It is still unknown if these gene products are initially responsible for the fast cell cycle progression or if the tumor's growth is the secondary cause of their upregulation (Denlinger & Thompson ,2012).



Targets that have been previously identified in other more prevalent types of carcinomas are the most clinically important molecular targets in esophageal cancer, and the significance of these proteins in esophageal cancer was later demonstrated. Clinical safety profiles provided by prior clinical studies of targeted drugs in the treatment of various cancers have made it easier for esophageal cancer to be treated with medication including VEGF, Her2/neu, and EGFR inhibitors. The development of drugs specifically suited for the treatment of Barrett's esophagus and esophageal adenocarcinomas may result from ongoing research into the particular pathophysiology involved in their development (Denlinger & Thompson, 2012).

Locating the Biomarkers for Barrett's Development into Adenocarcinoma

The clinical results of patients with Barrett esophagus may be significantly impacted by the early detection of those who are most at risk of developing adenocarcinoma or who have the illness in its early stages. As a deeper comprehension of the process behind the development of disease is created, potential biomarkers have been examined. As Barrett esophagus develops into cancer, inactivation of p16 is often observed,

which led to its investigation as a potential biomarker. The utility of p16 as a biomarker is limited because it is frequently suppressed in nonneoplastic tissue. Methylation of p16, HPP1, RUNX3, CDH13, TAC1, NELL1, AKAP12, AND SST were the only promoter methylation markers that were more common in patients who developed cancer than in those who did not, according to a retrospective double-blind study of biomarkers for esophageal adenocarcinoma. The biomarkers' respective sensitivity and specificity were 0.443 and 0.629 while maintaining a specificity of 0.9 or 0.8 (Denlinger & Thompson, 2012; Jin et al.,2009).Independent research is required to validate these findings, and the findings are not entirely reliable. They do, however, give us optimism that we will be able to create tests that would allow us to group patients based on their progression risks(Denlinger & Thompson, 2012).

Prospective therapies for GERD and Barrett's esophagus-related esophageal cancer

When the lining of the esophagus changes from its normal squamous epithelium to columnar epithelium, it is known as Barrett's esophagus. More current definitions of Barrett's esophagus stipulate that the columnar epithelium must be of the intestinal type, which means it must contain goblet cells, even if earlier definitions permitted any type of columnar epithelium (Sampliner,1998).In order to exclude intestinalized epithelium lengths that involved less than 3 cm of the esophagus, several researchers have defined Barrett esophagus according to a length criterion. Any length of intestinal metaplasia in the tubular esophagus is considered Barrett esophagus because even brief segments of intestinalized metaplasia raise the risk for esophageal cancer (Rudolph et al.,2000).Reflux symptoms are closely linked to Barrett's esophagus. Although less than 1% of people in the general population have Barrett esophagus, endoscopic investigations show that 5% to 15% of people who experience reflux symptoms for a long time will have some degree of Barrett esophagus (Menges et al.,2001; Csendes et al.,2000). Researchers have tried to stratify the risk for progression to esophageal cancer among patients with Barrett's esophagus,

as the illness may affect over 3 million people in the United States. Individuals with those risk factors may benefit from more rigorous endoscopic surveillance if trustworthy risk variables could be found. The degree of Barrett esophageal dysplasia, or precancerous cellular atypia, is currently the strongest predictive predictor. Adenocarcinoma at subsequent monitoring endoscopies is unlikely to occur in people without dysplasia, while it is more than 25% likely to occur in those with high-grade dysplasia (Reid et al.,2000). Moreover, there is a significant sample error in patients with high-grade dysplasia because of the random site of Barrett esophageal endoscopic biopsies. Up to half of the resection specimens from patients with high-grade dysplasia who have their esophagus removed show adenocarcinoma that has not been identified before. Because of this, a number of authority have recommended esophageal resection for patients with Barrett's esophagus and high-grade dysplasia. Others have proposed that people with Barrett esophagus and high-grade dysplasia undergo an intensive endoscopic surveillance protocol, with esophagectomy reserved for those who develop adenocarcinoma. They base this recommendation on the low incidence of incurable metastatic disease among those who develop cancer and are closely monitored with frequent surveillance endoscopies. To better define the best course of treatment for people with Barrett's esophagus who develop high-grade dysplasia, more longitudinal studies will be required (Schnell et al.,2001).Whether any medication, surgery, or endoscopic procedure may successfully restore Barrett's esophagus to its normal squamous epithelium is one area of growing therapeutic interest. When proton pump inhibitors were developed, doctors hypothesized that patients with Barrett's esophagus would benefit from severe acid suppression. Unfortunately, it is now known that proton pump inhibitor therapy does not completely prevent Barrett esophageal cancer from progressing to adenocarcinoma or significantly reduce the quantity of metaplastic tissue that is present. Therefore, the reduction of reflux symptoms is now the main reason for using proton pump inhibitors to treat patients with Barrett esophagus. There is no evidence to support the use of high doses of acid suppressants to treat

Barrett's esophagus patients in the hopes of lowering their risk of adenocarcinoma. It's unknown how surgical antireflux procedures relate to the management of Barrett's esophagus. 59 individuals with Barrett esophagus and no dysplasia participated in a randomized trial comparing medical and surgical antireflux therapy. Ortiz et al. found that the surgical group experienced a statistically significant reduction in the progression to any type of dysplasia, although the cancer rate decreased insignificantly. Among the 108 participants with Barrett esophagus who were monitored for an average of nearly 10 years, a randomized trial comparing medical and surgical antireflux therapy for reflux disease conducted in various US Veterans Affairs hospitals showed no statistically significant difference in the development of cancer between those treated medically and those treated surgically (4% vs. 3%). The study's sample size may have been insufficient to show such a difference, assuming one existed, according to the authors. Furthermore, a number of researchers have reported that adenocarcinoma can develop in the context of Barrett's esophagus in patients who had surgical antireflux operations months or years before the onset of cancer. Therefore, it is uncertain if surgical antireflux methods reduce the incidence of cancer, and patients who receive surgical treatment are still susceptible to adenocarcinoma. Barrett esophageal cancer risk reduction has been investigated using endoscopic ablation of Barrett esophageal regions. A number of endoscopic treatment methods, such as lasers, argon plasma coagulation, multipolar electrocoagulation, and photodynamic therapy, have been used to remove Barrett esophageal segments. It has been demonstrated that any of these methods can eliminate the metaplastic epithelium. After healing, the resulting epithelium is typically squamous when one or more of these techniques are used in conjunction with substantial acid suppression. However, islands of Barrett mucosa may remain in the treated area if the reversion to squamous epithelium is not complete. Additionally, what appears to be normal squamous epithelium may be covered with Barrett mucosa following endoscopic ablative therapy. Adenocarcinoma has been observed to grow beneath squamous mucosa in regions where there appears to be a return to squamous epithelium.

Consequently, it is still unknown how likely it is that regions of the Barrett esophagus treated with ablative therapy may develop cancer (Macey et al., 2001; Shand et al., 2001 and Van Laethem et al., 2001). These therapy approaches are now regarded as experimental, and it is unclear how useful they will be in the long run. These treatments might be most helpful for those with dysplasia who are at a higher risk for cancer, while those with Barrett esophagus and no dysplasia have a low chance of developing adenocarcinoma (Shaheen, & Ransohoff, 2002).

Conclusion

Esophageal adenocarcinoma (EAC) is becoming a more significant global health concern, especially because of its correlation with Barrett's esophagus and gastroesophageal reflux disease (GERD). Even while our knowledge of genetic and environmental risk factors has advanced, research on the human microbiome's function in EAC development is still in its infancy. Potential pathways for early identification and targeted therapy are suggested by the association between esophageal carcinogenesis and altered microbial populations.

The development of Barrett's esophagus into EAC is significantly influenced by GERD, mainly due to persistent inflammation and molecular alterations brought on by acid and bile reflux. Male sex, advanced age, obesity, smoking, and persistent GERD are risk factors for Barrett's esophagus and its development into cancer. The efficiency of current treatments, like as proton pump inhibitors and surgical procedures, in halting the advancement of cancer is still up for debate, despite their goal of managing GERD symptoms. New therapeutic approaches that hold promise for improved risk classification and individualized therapy include endoscopic procedures and the use of biomarkers. To completely comprehend the molecular processes involved in the shift from Barrett's esophagus to EAC and to enhance treatment procedures, more longitudinal research is necessary. To lessen the burden of this cancer in the interim, it is still essential to lead a healthy lifestyle, manage GERD well, and regularly check on high-risk individuals.

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