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Microbiome, innate Immunity, and esophageal adenocarcinoma

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Abstract

With the development of culture-independent technique, a complex microbiome has been established and described in the distal esophagus. Over recent decades, the incidence of esophageal adenocarcinoma (EAC)—a relatively rare cancer with high mortality—has increased dramatically in the United States. Several studies documenting an altered microbiome associated with EAC and its precedents (i.e., Barrett’s esophagus and reflux esophagitis) suggest that dysbiosis may be contributing to carcinogenesis, potentially mediated by interactions with toll-like receptors. Investigations attempting to associate viruses, in particular human papilloma virus, with EAC have not been as consistent. Regardless, currently available data is cross-sectional and therefore cannot prove causal relationships. Prospectively, microbiome studies open a new avenue to the understanding of the etiology and pathogenesis of reflux disorders and EAC.

Keywords

reflux; Barrett’s esophagus; adenocarcinoma; microbiome; chronic inflammation; viruses; bacteria; innate immunity

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Introduction

Esophageal adenocarcinoma (EAC) is a relatively rare but aggressive cancer that has been increasing in incidence, particularly among white males.^{12,3} EAC typically develops in the distal esophagus in response to mucosal injury, such as with exposure to gastric reflux, and is preceded by Barrett's esophagus (BE), a form of epithelial metaplasia. Beyond demographics, the major risk factors for EAC are gastroesophageal reflux disease (GERD), cigarette smoking, obesity, and low fruit and vegetable consumption. At a single cancer center, these risk factors represented a combined population attributable risk of nearly 80%.⁴

The relationship between GERD and EAC is complex. Symptomatic GERD is a strong risk factor for EAC,^{5,6} with increasing odds of an association as symptoms manifest for a longer duration or with increased frequency.⁷ However, esophagitis may develop in patients without significant acid exposure.⁸ The complications of GERD—esophagitis,⁹ BE,¹⁰ and EAC⁴—likewise may arise in the absence of preceding symptoms. Thus, acid reflux alone may not fully account for the pathogenesis of EAC. We hypothesize that characteristics of the esophageal microbiome facilitate the development of disease.

Microbiome of the Normal Esophagus

Early work to characterize an esophageal microbiome was undertaken by surgeons in the hope of preventing infections after thoracotomy.¹¹⁻¹⁴ These studies used conventional bacterial culture and therefore missed the majority of the indigenous esophageal biota, which is in a viable but non-culturable state.^{15,16} More recent approaches to characterize complex microbial communities have employed polymerase chain reaction (PCR) of 16S ribosomal RNA¹⁷ to better characterize non-culturable bacteria.

Using culture-independent technique to examine biopsies of normal esophagus, Pei *et al.* first described a complex bacterial biota in the distal esophagus.¹⁸ Ninety-five species were identified, including members of six phyla: Firmicutes, Bacteroides, Actinobacteria, Proteobacteria, Fusobacteria, and TM7. Two phyla seen in the oral cavity—*Spirochaetes* and *Defer-ribacteres*—were not present. Remarkably, findings were similar across specimens, suggesting a stable esophageal biota that is distinct from the flora of the oropharynx, stomach, or food bolus in transit. Microscopic examination of the tissue confirmed a close association of the bacteria with the cell surfaces of the mucosal epithelium *in situ*, suggesting a residential, rather than a transient, biota.

Microbiome in Disease States

In 2005, Pei *et al.*¹⁹ undertook the first study to apply cultivation-independent technique to the microbiome in esophageal disease, with the goal of demonstrating feasibility. Two 16S rRNA gene clones were recovered and examined from each of the esophageal biopsies taken from 24 subjects (9 with normal mucosa, 12 with GERD, and 3 with BE). As expected, bacterial signals were successfully detected in all biopsies, and the overall diversity and community membership resembled those of the normal esophageal microbiome.

A more comprehensive approach was taken by Yang *et al.*²⁰ in 2009. Representing one of the largest human microbiome studies to date, a total of 6,800 16S rRNA gene clones from 34 subjects were analyzed by Sanger sequencing. Using both unsupervised and phenotype-guided clustering analyses, samples were found to contain one of two distinct microbiomes. Microbiome type I was mainly associated with normal esophagus and was predominated by gram-positive bacteria from the *Firmicutes* phylum, of which *Streptococcus* was the most dominant genus. Microbiome type II had greater proportion of gram-negative anaerobes/microaerophiles (phyla *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Spirochaetes*) and primarily correlated with RE (odds ratio, 15.4) and BE (odds ratio, 16.5). The microbiome did not differ between GERD and BE patients.

In a subsequent study from Japan, Liu *et al.*²¹ compared the bacterial populations present in biopsies taken from 18 Japanese subjects (6 each with normal esophagus, RE, and BE). A unique test performed was quantification of total bacterial loads by 16S rRNA PCR. Notably, about 10^6 - 10^7 colony forming units were counted in each sample, irrespective of disease. This finding suggests that changes in the relative abundance of taxa, rather than differences in absolute bacterial loads, are likely more relevant to esophageal diseases. Although far from comprehensive (only approximately 24 clones were sequenced per sample), *Veillonella* (19%), *Prevotella* (12%), *Neisseria* (4%), and *Fusobacterium* (9%) were found to be prevalent in patients with RE and BE but were not detected in controls. These observations support the earlier work of Pei¹⁹ and Yang²⁰, confirming that the esophageal microbiome is reliably altered in reflux disorders (**Figure 1**).

Influence of Toll-Like Receptors

Toll-like receptors (TLRs) expressed in the microenvironment of the esophageal mucosa mediate the interaction of the immune system with the microbiome. TLRs coordinate between a state of homeostasis and a state of injury.²² Thus, TLRs have become an area of interest as potential mediators of inflammation-related carcinogenesis.²³ In particular, TLR3, TLR4, TLR5, and TLR9 have been suggested as potential mediators of the progression from reflux disorders to EAC (**Table 1**).

TLR3 and TLR4

TLR3²⁴ and TLR4²⁵ have been implicated in GERD-spectrum disorders largely by documentation of their expression and expression of their downstream products—cyclooxygenase-2 (COX-2), interleukin-8 (IL-8), nuclear factor- κ B (NF- κ B), and nitric oxide (NO)—in tissue samples and *ex vivo* cell culture²⁶⁻³². In a murine model, inhibition of COX-2 reduced progression of BE to EAC.³³ In biopsies of RE, higher levels of IL-8 are associated with dysplasia and EAC,^{34,35} as well as recurrence of symptoms after cessation of acid-reducing therapy.³⁶ NF- κ B is considered a promoter of inflammation-associated carcinogenesis³⁷ and mediates the initial metaplastic changes that lead to BE.³⁸ Blockade of NF- κ B activity has been shown to reduce the acid-induced inflammatory response in cell lines derived from EAC.³⁹ In mice, TLR4-mediated release of NO by colon cancer cells treated with lipopolysaccharide (LPS) has been shown to suppress cytotoxic T-cell and natural killer cell activity, promoting tumor growth and shortening mouse survival.⁴⁰ NO

release has been suggested as an explanation for LPS-induced dysfunction of the lower esophageal sphincter.⁴¹

Thus, a body of evidence is mounting to suggest a role for TLR3 and TLR4 in the pathogenesis of EAC. One of the exogenous ligands for TLR4 is lipopolysaccharide (LPS), a component of the cell membrane of gram-negative bacteria.⁴² Based on the findings of Yang,²⁰ it can be proposed that a shift in the esophageal microbiome in GERD-spectrum disorders towards a predominance of gram-negative bacteria might preferentially stimulate TLR4, triggering a larger and more carcinogenic inflammatory cascade.

Unfortunately, the *in situ* ligand for TLR3 remains unidentified, making a plausible biological pathway difficult to hypothesize. The natural ligand for TLR3 is viral double-stranded DNA, but no virus—with the possible exception of human papilloma virus (HPV)—has been identified as playing a consistent role in GERD-spectrum disorders.

TLR5 and TLR9

Less evidence is available to support roles for TLR5 or TLR9 in the development of EAC. In a case series from a single medical center, TLR5 expression within the esophageal epithelium was shown to increase in a stepwise manner with progression from normal to dysplastic and eventually neoplastic states.⁴³ Meanwhile, TLR9, when strongly expressed by EAC, has been associated with markers of poor prognosis—advanced stage, high grade pathology, tumor unresectability, lymph node involvement, and distant metastases—as well as shortened survival.⁴⁴

Therefore, more research will need to be performed before a plausible role in for TLR5 and TLR9 in the pathogenic sequence can be hypothesized. The ligands for TLR5 and TLR9 are bacterial flagellin⁴⁵ and bacterial DNA,⁴⁶ respectively. Thus, though the esophageal microbiome likely plays a role in their activation, it is unclear how the altered microbiome documented by Yang²⁰ might interact with TLR5 and TLR9 differently than the normal one.

Roles of Viruses

HPV is the subject of interest as a potential driver of tumorigenesis in the distal esophagus. HPV is known to have a strong association with esophageal squamous cell carcinoma,⁴⁷ but its role in the development of EAC is much less clear. Outside of the esophagus, HPV has an established role in the development of cancers of the cervix⁴⁸ and oropharynx.⁴⁹ Analogies have been drawn between the pathogenesis of cervical cancer and EAC,⁵⁰ based in part on similar premalignant changes in the epithelial cell expression of human leukocyte antigen (HLA) that are suspected to affect the immune response to HPV.⁵¹ However, investigations attempting to document HPV in EAC and its premalignant conditions have yielded mixed results.⁵²

In the largest study to address this topic, Rajendra *et al.*⁵³ evaluated biopsies from 261 Australian patients by PCR for HPV DNA. HPV, when identified, was evaluated for transcriptional activity by quantification of oncogene mRNA expression and protein

immunohistochemistry. 24 of 35 (68.6%) patients with dysplastic BE and 18 of 27 (66.7%) with EAC were found to be HPV-positive, compared with 17 of 77 (22.1%) patients with non-dysplastic BE and 22 of 122 (18.0%) controls. The majority of HPV strains detected were high risk subtypes (16 and 18), with markers for transcriptional activity found predominantly in dysplastic and malignant samples. Though the investigators noted that the background prevalence of oral HPV is higher in Australians⁵⁴ than Americans,⁵⁵ these data support a potential role for HPV in the pathway from metaplasia to dysplasia and neoplasia in the esophagus.

The only other study to show a robust association between BE and HPV is a biopsy series from Mexico.⁵⁶ HPV was detected in 27 of 28 (96%) biopsies of BE, compared with only 6 of 23 (26%) samples with esophagitis. However, samples were not stratified by dysplasia, and no controls were available for comparison. Regardless, these data seem to suggest that HPV is, at the very least, not uncommon in the setting of distal esophageal metaplasia.

Other studies have failed to show a similar connection. In an American biopsy series,⁵⁷ HPV DNA was detected in 23 of 84 (27.4%) BE cases, 11 of 36 (31%) cases of EAC, and in 7 of 29 (24%) normal controls. No statistical differences were found between groups with regards to the presence of HPV, the presence of high- vs low-risk subtypes, or immunohistochemistry for P16INK4a (a viral product that is used as a marker for activity of infection). However, only 6 of the cases of BE evaluated were dysplastic, which may explain the different findings in this study when compared with those of Rajendra (**Table 2**).⁵⁸

Still more investigations from America have shown HPV to be entirely absent from biopsies of non-dysplastic BE⁵⁹ or frozen surgical specimens from resected EAC. In a study from the UK, HPV was identified in only 1 of 73 biopsies of BE.⁶⁰ In an Italian biopsy series, HPV DNA was detected in 2 of 20 (10%) cases of EAC, compared with 8 of 27 (30%) cases of RE.⁶¹ These results are difficult to reconcile with those of Acevedo-Nuno⁵⁵ and suggest that HPV is not common in EAC and its precursors. Though background geographical variation in prevalence of HPV may explain these disparities, the low frequency of HPV in American studies stands in stark contrast to the rising incidence of EAC over recent decades in the United States.

Regarding other viruses, a study evaluating surgical frozen sections by PCR for adenovirus, CMV, and EBV failed to show any differences between patients with EAC and controls.⁶²

Helicobacter pylori

Meta-analyses and cross-sectional evaluation of biopsies have shown an inverse relationship between the presence of *H. pylori* and GERD-spectrum disorders.⁶³⁻⁶⁵ However, eradication of *H. pylori* does not induce new cases of GERD, nor does it worsen GERD symptoms (except in patients with hiatal hernia and corpus gastritis).⁶⁶ The role of *H. pylori* in the pathogenesis of GERD, BE and EAC remains an unclear and controversial topic that has been extensively reviewed elsewhere.⁶⁷

Potential Role of the Microbiome in Disease

Though the microbiome has been implicated in inflammation and carcinogenesis elsewhere in the gastrointestinal tract,⁶⁸ studies to date of the distal esophagus have been cross-sectional and therefore unable to establish a causal relationship. Given that the gut microbiome has been shown to be heritable,⁶⁹ it is unclear whether the variant microbiome demonstrated by Yang²⁰ was acquired in response to environmental factors—such as antibiotics⁷⁰—was deposited directly by gastric reflux, or is stable from childhood. Likewise, it cannot be determined whether this variant microbiome caused disease by induction of abnormal lower esophageal sphincter function, accelerated disease by potentiating inflammation via interaction with TLRs, predisposed towards disease by altering the immune response to incipient cancer, or resulted from changes in the local microenvironment related to acid exposure. Longitudinal studies will be necessary to parse out temporal relationships.

Perspectives

Esophageal microbiology is an understudied field, especially in EAC. Prospective studies to explore whether the microbiome changes before or after onset of disease are the next logical step to evaluate causality. A large scale study on this topic has been funded under the NIH Human Microbiome Project and may fill the knowledge gap. Large cohorts, such as the National Cancer Institute-Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial Cohort (NCI-PLCO) and the American Cancer Society Cancer Prevention Study II Cohort (ACS-CPS-II), have access to tissue samples collected prior to cancer diagnosis and therefore should prove invaluable to further characterize the role of the esophageal microbiome in carcinogenesis.⁷¹

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Reference

1. Devesa SS, Blot WJ, Fraumeni JF Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer*. 1998; 83:2049–53. [PubMed: 9827707]
2. Daly JM, Karnell LH, Menck HR. National Cancer Data Base report on esophageal carcinoma. *Cancer*. 1996; 78:1820–8. [PubMed: 8859198]
3. Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. *Journal of the National Cancer Institute*. 2008; 100:1184–7. [PubMed: 18695138]
4. Engel LS, Chow WH, Vaughan TL, et al. Population attributable risks of esophageal and gastric cancers. *Journal of the National Cancer Institute*. 2003; 95:1404–13. [PubMed: 13130116]
5. Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *New Engl J Med*. 1999; 340:825–31. [PubMed: 10080844]
6. Rubenstein JH, Taylor JB. Meta-analysis: the association of oesophageal adenocarcinoma with symptoms of gastro-oesophageal reflux. *Alimentary pharmacology & therapeutics*. 2010; 32:1222–7. [PubMed: 20955441]

7. Farrow DC, Vaughan TL, Sweeney C, et al. Gastroesophageal reflux disease, use of H-2 receptor antagonists, and risk of esophageal and gastric cancer. *Cancer Cause Control*. 2000; 11:231–8.
8. Schlesinger PK, Donahue PE, Schmid B, Layden TJ. Limitations of 24-hour intraesophageal pH monitoring in the hospital setting. *Gastroenterology*. 1985; 89:797–804. [PubMed: 4029559]
9. Zentilin P, Savarino V, Mastracci L, et al. Reassessment of the diagnostic value of histology in patients with GERD, using multiple biopsy sites and an appropriate control group. *Am J Gastroenterol*. 2005; 100:2299–306. [PubMed: 16181384]
10. Gerson LB, Shetler K, Triadafilopoulos G. Prevalence of Barrett's esophagus in asymptomatic individuals. *Gastroenterology*. 2002; 123:461–7. [PubMed: 12145799]
11. Finlay IG, Wright PA, Menzies T, McArdle CS. Microbial flora in carcinoma of oesophagus. *Thorax*. 1982; 37:181–4. [PubMed: 7101222]
12. Bricard H, Deshayes JP, Sillard B, et al. [Antibiotic prophylaxis in surgery of the esophagus]. *Annales francaises d'anesthesie et de reanimation*. 1994; 13:S161–8.
13. Gagliardi D, Makihara S, Corsi PR, et al. Microbial flora of the normal esophagus. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / ISDE*. 1998; 11:248–50.
14. Mannell A, Plant M, Frolich J. The microflora of the oesophagus. *Annals of the Royal College of Surgeons of England*. 1983; 65:152–4. [PubMed: 6859774]
15. Oliver JD. The viable but nonculturable state in bacteria. *J Microbiol*. 2005; 43:93–100. Spec No. [PubMed: 15765062]
16. Oliver JD. Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS microbiology reviews*. 2010; 34:415–25. [PubMed: 20059548]
17. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*. 1991; 173:697–703. [PubMed: 1987160]
18. Pei Z, Bini EJ, Yang L, Zhou M, Francois F, Blaser MJ. Bacterial biota in the human distal esophagus. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:4250–5. [PubMed: 15016918]
19. Pei Z, Yang L, Peek RM Jr, Levine SM, Pride DT, Blaser MJ. Bacterial biota in reflux esophagitis and Barrett's esophagus. *World journal of gastroenterology : WJG*. 2005; 11:7277–83. [PubMed: 16437628]
20. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology*. 2009; 137:588–97. [PubMed: 19394334]
21. Liu N, Ando T, Ishiguro K, et al. Characterization of bacterial biota in the distal esophagus of Japanese patients with reflux esophagitis and Barrett's esophagus. *BMC infectious diseases*. 2013; 13:130. [PubMed: 23496929]
22. Gribar SC, Richardson WM, Sodhi CP, Hackam DJ. No longer an innocent bystander: Epithelial toll-like receptor signaling in the development of mucosal inflammation. *Mol Med*. 2008; 14:645–59. [PubMed: 18584047]
23. Ioannou S, Voulgarelis M. Toll-Like Receptors, Tissue Injury, and Tumorigenesis. *Mediat Inflamm*. 2010
24. Mulder DJ, Lobo D, Mak N, Justinich CJ. Expression of toll-like receptors 2 and 3 on esophageal epithelial cell lines and on eosinophils during esophagitis. *Digestive diseases and sciences*. 2012; 57:630–42. [PubMed: 21960283]
25. Verbeek RE, Siersema PD, Ten Kate FJ, et al. Toll-like receptor 4 activation in Barrett's esophagus results in a strong increase in COX-2 expression. *Journal of gastroenterology*. 2013
26. Wilson KT, Fu S, Ramanujam KS, Meltzer SJ. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. *Cancer Res*. 1998; 58:2929–34. [PubMed: 9679948]
27. Shirvani VN, Ouatu-Lascar R, Kaur BS, Omary MB, Triadafilopoulos G. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: Ex vivo induction by bile salts and acid exposure. *Gastroenterology*. 2000; 118:487–96. [PubMed: 10702199]

28. Lim DM, Narasimhan S, Michaylira CZ, Wang ML. TLR3-mediated NF- κ B signaling in human esophageal epithelial cells. *American journal of physiology Gastrointestinal and liver physiology*. 2009; 297:G1172–80. [PubMed: 19779021]
29. Fitzgerald RC, Onwuegbusi BA, Bajaj-Elliott M, Saeed IT, Burnham WR, Farthing MJ. Diversity in the oesophageal phenotypic response to gastro-oesophageal reflux: immunological determinants. *Gut*. 2002; 50:451–9. [PubMed: 11889061]
30. Isomoto H, Saenko VA, Kanazawa Y, et al. Enhanced expression of interleukin-8 and activation of nuclear factor kappa-B in endoscopy-negative gastroesophageal reflux disease. *Am J Gastroenterol*. 2004; 99:589–97. [PubMed: 15089887]
31. Isomoto H, Wang A, Mizuta Y, et al. Elevated levels of chemokines in esophageal mucosa of patients with reflux esophagitis. *Am J Gastroenterol*. 2003; 98:551–6. [PubMed: 12650786]
32. Yoshida N, Uchiyama K, Kuroda M, et al. Interleukin-8 expression in the esophageal mucosa of patients with gastroesophageal reflux disease. *Scandinavian journal of gastroenterology*. 2004; 39:816–22. [PubMed: 15513378]
33. Buttar NS, Wang KK, Leontovich O, et al. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. *Gastroenterology*. 2002; 122:1101–12. [PubMed: 11910360]
34. Oh DS, DeMeester SR, Vallbohmer D, et al. Reduction of interleukin 8 gene expression in reflux esophagitis and Barrett's esophagus with antireflux surgery. *Arch Surg*. 2007; 142:554–9. discussion 9–60. [PubMed: 17576892]
35. O'Riordan JM, Abdel-latif MM, Ravi N, et al. Proinflammatory cytokine and nuclear factor kappa-B expression along the inflammation-metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. *Am J Gastroenterol*. 2005; 100:1257–64. [PubMed: 15929754]
36. Isomoto H, Inoue K, Kohno S. Interleukin-8 levels in esophageal mucosa and long-term clinical outcome of patients with reflux esophagitis. *Scandinavian journal of gastroenterology*. 2007; 42:410–1. [PubMed: 17354124]
37. Maeda S, Omata M. Inflammation and cancer: role of nuclear factor-kappaB activation. *Cancer science*. 2008; 99:836–42. [PubMed: 18294278]
38. Souza RF, Krishnan K, Spechler SJ. Acid, bile, and CDX: the ABCs of making Barrett's metaplasia. *American journal of physiology Gastrointestinal and liver physiology*. 2008; 295:G211–8. [PubMed: 18556417]
39. Zhou X, Li D, Resnick MB, Wands J, Cao W. NADPH oxidase NOX5-S and nuclear factor kappaB1 mediate acid-induced microsomal prostaglandin E synthase-1 expression in Barrett's esophageal adenocarcinoma cells. *Molecular pharmacology*. 2013; 83:978–90. [PubMed: 23439561]
40. Huang B, Zhao J, Li H, et al. Toll-like receptors on tumor cells facilitate evasion of immune surveillance. *Cancer Res*. 2005; 65:5009–14. [PubMed: 15958541]
41. Fan YP, Chakder S, Gao F, Rattan S. Inducible and neuronal nitric oxide synthase involvement in lipopolysaccharide-induced sphincteric dysfunction. *American journal of physiology Gastrointestinal and liver physiology*. 2001; 280:G32–42. [PubMed: 11123195]
42. Hoshino K, Takeuchi O, Kawai T, et al. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: Evidence for TLR4 as the Lps gene product. *J Immunol*. 1999; 162:3749–52. [PubMed: 10201887]
43. Helminen O, Huhta H, Takala H, et al. Increased Toll-like receptor 5 expression indicates esophageal columnar dysplasia. *Virchows Archiv : an international journal of pathology*. 2014; 464:11–8. [PubMed: 24221343]
44. Kauppila JH, Takala H, Selander KS, Lehenkari PP, Saarnio J, Karttunen TJ. Increased Toll-like receptor 9 expression indicates adverse prognosis in oesophageal adenocarcinoma. *Histopathology*. 2011; 59:643–9. [PubMed: 22014045]
45. Hayashi F, Smith KD, Ozinsky A, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*. 2001; 410:1099–103. [PubMed: 11323673]
46. Hemmi H, Takeuchi O, Kawai T, et al. A Toll-like receptor recognizes bacterial DNA. *Nature*. 2000; 408:740–5. [PubMed: 11130078]

47. Ding GC, Ren JL, Chang FB, et al. Human papillomavirus DNA and P16(INK4A) expression in concurrent esophageal and gastric cardia cancers. *World journal of gastroenterology : WJG*. 2010; 16:5901–6. [PubMed: 21155014]
48. Bosch FX, Lorincz A, Munoz N, Meijer CJLM, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol*. 2002; 55:244–65. [PubMed: 11919208]
49. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *The New England journal of medicine*. 2007; 356:1944–56. [PubMed: 17494927]
50. Rajendra S, Robertson IK. Similar immunogenetics of Barrett's oesophagus and cervical neoplasia: is HPV the common denominator? *J Clin Pathol*. 2010; 63:1–3. [PubMed: 20026700]
51. Rajendra S, Ackroyd R, Karim N, Mohan C, Ho JJ, Kutty MK. Loss of human leucocyte antigen class I and gain of class II expression are early events in carcinogenesis: clues from a study of Barrett's oesophagus. *J Clin Pathol*. 2006; 59:952–7. [PubMed: 16467164]
52. Wang X, Tian X, Liu F, et al. Detection of HPV DNA in esophageal cancer specimens from different regions and ethnic groups: a descriptive study. *BMC cancer*. 2010; 10:19. [PubMed: 20078887]
53. Rajendra S, Wang B, Snow ET, et al. Transcriptionally Active Human Papillomavirus Is Strongly Associated With Barrett's Dysplasia and Esophageal Adenocarcinoma. *Am J Gastroenterol*. 2013; 108:1082–93. [PubMed: 23588239]
54. Sedlak-Weinstein, LRT. Prevalence of HPV in suspicious oral lesions. The 47th Annual Scientific Meeting of the Australia and New Zealand Division of the International Association of Dental Research; 2007.
55. Gillison ML, Broutian T, Pickard RK, et al. Prevalence of oral HPV infection in the United States, 2009–2010. *JAMA : the journal of the American Medical Association*. 2012; 307:693–703.
56. Acevedo-Nuno E, Gonzalez-Ojeda A, Vazquez-Camacho G, Balderas-Pena Luz Ma A, Moreno-Villa H, Montoya-Fuentes H. Human papillomavirus DNA and protein in tissue samples of oesophageal cancer, Barrett's oesophagus and oesophagitis. *Anticancer research*. 2004; 24:1319–23. [PubMed: 15154668]
57. Iyer A, Rajendran V, Adamson CSC, Peng Z, Cooper K, Evans MF. Human papillomavirus is detectable in Barrett's esophagus and esophageal carcinoma but is unlikely to be of any etiologic significance. *J Clin Virol*. 2011; 50:205–8. [PubMed: 21169053]
58. El-Serag HB, Hollier JM, Gravitt P, Alsarraj A, Younes M. Human papillomavirus and the risk of Barrett's esophagus. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / ISDE*. 2013; 26:517–21.
59. Kamath AM, Wu TT, Heitmiller R, Daniel R, Shah KV. Investigation of the association of esophageal carcinoma with human papillomaviruses. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / ISDE*. 2000; 13:122–4.
60. Rai N, Jenkins GJ, McAdam E, Hibbitts SJ, Fiander AN, Powell NG. Human papillomavirus infection in Barrett's oesophagus in the UK: an infrequent event. *J Clin Virol*. 2008; 43:250–2. [PubMed: 18718811]
61. Tornesello ML, Monaco R, Nappi O, Buonaguro L, Buonaguro FM. Detection of mucosal and cutaneous human papillomaviruses in oesophagitis, squamous cell carcinoma and adenocarcinoma of the oesophagus. *J Clin Virol*. 2009; 45:28–33. [PubMed: 19307150]
62. Morgan RJ, Perry AC, Newcomb PV, Hardwick RH, Alderson D. Investigation of oesophageal adenocarcinoma for viral genomic sequences. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology*. 1997; 23:24–9.
63. Rokkas T, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. Relationship between *Helicobacter pylori* infection and esophageal neoplasia: A meta-analysis. *Clin Gastroenterol H*. 2007; 5:1413–7.
64. Islami F, Kamangar F. *Helicobacter pylori* and esophageal cancer risk: a meta-analysis. *Cancer Prev Res (Phila)*. 2008; 1:329–38. [PubMed: 19138977]

65. Loffeld RJ, Werdmuller BF, Kuster JG, Perez-Perez GI, Blaser MJ, Kuipers EJ. Colonization with cagA-positive *Helicobacter pylori* strains inversely associated with reflux esophagitis and Barrett's esophagus. *Digestion*. 2000; 62:95–9. [PubMed: 11025356]
66. Hamada H, Haruma K, Mihara M, et al. High incidence of reflux oesophagitis after eradication therapy for *Helicobacter pylori*: impacts of hiatal hernia and corpus gastritis. *Alimentary pharmacology & therapeutics*. 2000; 14:729–35. [PubMed: 10848656]
67. Peek RM. *Helicobacter pylori* and Gastroesophageal Reflux Disease. Current treatment options in gastroenterology. 2004; 7:59–70. [PubMed: 14723839]
68. Kado S, Uchida K, Funabashi H, et al. Intestinal microflora are necessary for development of spontaneous adenocarcinoma of the large intestine in T-cell receptor beta chain and p53 double-knockout mice. *Cancer Res*. 2001; 61:2395–8. [PubMed: 11289103]
69. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:11070–5. [PubMed: 16033867]
70. Ferrer M, Martins dos Santos VA, Ott SJ, Moya A. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. *Gut microbes*. 2014; 5:64–70. [PubMed: 24418972]
71. Hayes RB, Reding D, Kopp W, et al. Etiologic and early marker studies in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. *Controlled clinical trials*. 2000; 21:349S–55S. [PubMed: 11189687]

Key Points

1. With the development of culture-independent technique, a complex microbiome has been established and described in the distal esophagus.
2. Over recent decades, the incidence of esophageal adenocarcinoma (EAC)—a relatively rare cancer with high mortality—has increased dramatically in the United States.
3. Several studies documenting an altered microbiome associated with EAC and its precedents (i.e., Barrett's esophagus and reflux esophagitis) suggest that dysbiosis may be contributing to carcinogenesis, potentially mediated by interactions with toll-like receptors.
4. Investigations attempting to associate viruses, in particular human papilloma virus, with EAC have not been as consistent. Regardless, currently available data is cross-sectional and therefore cannot prove causal relationships.
5. Prospectively, microbiome studies open a new avenue to the understanding of the etiology and pathogenesis of reflux disorders and EAC.

Figure 1.
Timeline of esophageal microbiology

Table 1

Summary of studies linking TLRs to GERD, BE and EAC

Category	TLR-3	TLR-4	TLR-5	TLR-9
Function	Intracellular. Recognizes viral dsRNA and induces the activation of NF- κ B and production of Type 1 interferons.	Cell surface. Implicated in signal transduction events induced by LPS found in most gram-negative bacteria.	Cell surface. Protein product recognizes bacterial flagellin. Activation of this receptor mobilizes NF- κ B.	Intracellular. Recognizes unmethylated CpG sequences in DNA molecules.
Disease	Esophagitis, GERD	BE	BE, EAC	EAC
Specimen	Biopsy	Biopsy	Biopsy	Biopsy
Results	Increased TLR-3 expression on EAC derived epithelial cell lines (HET-1A, TE-1 and TE-7) TLR-2+ immune cells but not TLR-2 expression, were seen in biopsies from patients with both GERD and eosinophilic esophagitis	NF- κ B activation upon TLR-4 stimulation Increased IL-8 expression and activation upon TLR-4 activation Increased COX-2 expression in BE upon TLR-4 activation	TLR-5 overexpression was a sensitive and specific marker in identification of dysplastic lesions in BE Dysplastic lesions and adenocarcinomas showed increased TLR-5 expression TLR-5 had no prognostic value in EAC	TLR-9 was expressed in all EAC tumors. High TLR-9 expression correlated with advanced tumor stage, tumor unresectability, poor differentiation and high proliferation. Cumulative 10-year survival for EAC patients with weak TLR-9 expression was 35.2% and 9.3% respectively for patients with strong TLR-9 expression
Reference	24	25	43	44

Data From Refs 24, 25, 43, 44

Table 2

A summary of previous studies that examined human papillomavirus (HPV) infection in Barrett's esophagus (BE) or esophageal adenocarcinoma (EAC)

Study first author (year)	Region	Cases <i>n</i> (BE/EAC)	Case tissue examined (design)	Control subjects <i>n</i>	Control tissue examined	Method of HPV testing	% HPV positive
Acevedo-Nuno E <i>et al.</i> (2004) ⁵⁵	Mexico	45 (28/17)	Lesion-targeted biopsy	23 esophagitis	Gastroesophageal junction	PCR ^b	96% BE, 26% controls ($P < 0.01$).
						IHC ^c	increasing correlation with esophagitis, BE, and EAC ($P = 0.000$)
Rai N <i>et al.</i> (2008) ⁵⁹	United Kingdom	73 (73/0)	Biopsy from suspected BE lesion	None	N/A ^d	PCR	1.4% in BE
Wang X <i>et al.</i> (2010) ⁵²	United States	34 (0/34)	Tumor site	54 ESCC ^a biopsies	Tumor site	PCR	52.9% in EAC versus 66.7% in ESCC ($P=0.2$)
Iyer A <i>et al.</i> (2011) ⁵⁷	North America	116 (80/36)	Lesion-targeted biopsy	29 normal biopsies	Biopsy at gastroesophageal junction	PCR	28% BE, 31% EAC, 21% control

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^aESCC, esophageal squamous cell carcinoma;

^bPCR, polymerase chain reaction;

^cIHC, immunohistochemistry;

^dN/A, not applicable.