

REVIEW

The Immune Underpinnings of Barrett's-Associated Adenocarcinogenesis: a Retrial of Nefarious Immunologic Co-Conspirators



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There is no doubt that chronic gastroesophageal reflux disease increases the risk of esophageal adenocarcinoma (EAC) by several fold (odds ratio, 6.4; 95% CI, 4.6–9.1), and some relationships between reflux disease-mediated inflammation and oncogenic processes have been explored; however, the precise interconnections between the immune response and genomic instabilities underlying these pathologic processes only now are emerging. Furthermore, the precise cell of origin of the precancerous stages associated with EAC development, Barrett's esophagus, be it cardia resident or embryonic remnant, may shape our interpretation of the likely immune drivers. This review integrates the current collective knowledge of the immunology underlying EAC development and outlines a framework connecting proinflammatory pathways, such as those mediated by interleukin 1 β , tumor necrosis factor α , leukemia inhibitory factor, interleukin 6, signal transduction and activator of transcription 3, nuclear factor- κ B, cyclooxygenase-2, and transforming growth factor β , with oncogenic pathways in the gastroesophageal reflux disease-Barrett's esophagus-EAC cancer sequence. Further defining these immune and molecular railroads may show a map of the routes taken by gastroesophageal cells on their journey toward EAC tumor phylogeny. The selective pressures applied by this immune-induced journey likely impact the phenotype and genotype of the resulting oncogenic destination and further exploration of lesser-defined immune drivers may be useful in future individualized therapies or enhanced selective application of recent immune-driven therapeutics. (*Cell Mol Gastroenterol Hepatol* 2022;13:1297-1315; <https://doi.org/10.1016/j.jcmgh.2022.01.023>)

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Esophageal adenocarcinoma (EAC) is clinically relevant as a result of its increasing disease demographic, high mortality rate (<20% 5-year survival), and lack of effective and early prescreening methods. Although EAC has affected mainly Western nations, the global spread of a high-calorie, cholesterol-rich diet has increased the occurrence of both abdominal obesity and gastroesophageal

reflux disease (GERD): 2 factors that increase the risk of EAC by several-fold.¹ For example, demographics with traditionally low EAC to esophageal squamous cell carcinoma (ESCC) incidence, such as those in several East Asian countries, have seen a progressive increase in the EAC/ESCC ratio that is parallel to the increase in obesity.¹ Thus, the study of EAC pathogenesis and its treatment remains a pressing issue. Genome-wide association studies have defined polymorphic variation associated with GERD, Barrett's esophagus (BE), and EAC development.^{2–4} These data recently were used to show that one of the most significantly genetically correlated phenotypes with GERD was BE/EAC, along with depression and education, using Linkage disequilibrium (LD) score regression analyses.^{2,5}

The transient yet long-term exposure of esophageal tissue by gastric contents resulting from GERD, in addition to the accompanying immune response, has been theorized as a key developmental driver of BE, a potentially premalignant condition highly associated with EAC. As a type of intestinal metaplasia, BE is characterized by the replacement of stratified squamous epithelium with simple columnar epithelium that overlies mucous-secreting glands. Evidence from animal models, human cell lines, and patient-derived tissues has shown several potential cellular origins of BE. These include esophageal progenitor cells from either the basal squamous epithelium or in ductal cells of submucosal glands, squamocolumnar junction, gastric cardia, or bone

Abbreviations used in this paper: BE, Barrett's esophagus; COX2, cyclooxygenase-2; C1q, complement component 1q; EAC, esophageal adenocarcinoma; EoE, eosinophilic esophagitis; ESCC, esophageal squamous cell carcinoma; GATA6, GATA binding protein 6; GDF, growth differentiation factor; GEJ, gastroesophageal junction; GERD, gastroesophageal reflux disease; HGD, high-grade dysplasia; IFN- γ , interferon γ ; κ B, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; IL, interleukin; JAK3, Janus kinase 3; LIF, leukemia inhibitory factor; MDSC, myeloid-derived suppressor cell; NF- κ B, nuclear factor- κ B; NK, natural killer; PGE2, prostaglandin E2; PPI, proton pump inhibitor; SCJ, squamocolumnar junction; SMAD, small mothers against decapentaplegic; STAT, signal transduction and activator of transcription; SYK, Spleen tyrosine kinase; TAM, tumor-associated macrophage; TGF β , transforming growth factor β ; Th, T-helper cell; TME, tumor microenvironment; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor.



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marrow⁶⁻²²; however, despite several decades of vigorous scientific inquiry, the origin of BE cells remains unknown. Regardless of its source, BE has been shown to differentiate into dysplastic populations over time as the highly inflammatory, GERD-induced environment ultimately selects for the expansion of highly proliferative and apoptotic-resistant clones. As the accumulation of precancerous mutations, such as loss of Tumor Protein P53 (TP53) and Cyclin dependent kinase inhibitor 2A (CDKN2A), persist in certain dysplastic populations, these populations may undergo tumorigenesis and subsequent metastasis.²³⁻²⁵ Metaplastic transformation of the gastroesophageal junction is linked consistently with the reflux of acidified bile acid and much of the initial research focused on the ability of acidified bile acid to induce the expression of intestine phenotype-associated genes such as mucins or keratins. Although the caustic damage from sustained acid/bile acid reflux plays a significant part in the selection of precancerous BE cells, the delayed immune response that follows injury may play a much more significant role in shaping the molecular landscape underlying GERD-BE-EAC transitions. For example, Souza et al²⁶ showed that esophageal cell lines exposed to acidic bile did not show immediate caustic injury, but rather a delayed inflammatory immune response. In addition, many of these inflammatory factors, mostly cytokines, may act as the molecular crossroads that connect the immunologic, metaplastic, and metastatic railways that form the tumorigenic path toward EAC. Of these pathways, the major molecules of interest in this review are interleukin (IL)1 β , tumor necrosis factor α (TNF α), nuclear factor- κ B (NF- κ B), leukemia inhibitory factor (LIF), IL6, signal transduction and activator of transcription (STAT)3, cyclooxygenase-2 (COX2) and associated prostaglandins, transforming growth factor β (TGF β), and complement proteins. This review will contextualize and integrate these pathways in chronological order from a primarily immunologic standpoint, concluding with a discussion on how these perspectives aid the development of personalized oncogenomic treatments targeting key dysregulated pathways central to the pathogenesis of EAC.

In the Beginning: Innate IL1 β Activation

IL1 β is a proinflammatory cytokine that plays an important role in the initiation of the innate immune response.²⁷⁻³⁰ Pattern recognition receptors such as Toll-like receptors, retinoic acid inducible gene-I (RIG-1) like receptors, and nucleotide-binding oligomerization domain-like receptors are important in the innate activation of IL1 β .²⁷⁻³¹ The cleavage of pro-IL1 β by caspase-1 is required for the formation of active IL1 β , which then may induce consequent proinflammatory IL6 and IL8 expression through NF- κ B-mediated signaling (Figure 1). Dysregulated IL1 β signaling, caused by genetically derived aberrations of the inflammasome complex or cellular exposure to chronic inflammatory agents, is a hallmark of several diseases such as inflammatory bowel disease, type 2 diabetes, and

obesity.^{27,32-34} IL1 β also has been implicated in the progression of several cancers, including gastric, pancreatic ductal adenocarcinoma, breast cancer, and lung adenocarcinoma.^{27,32-36} Analysis of polymorphic variation surrounding the *IL1B* gene along the esophageal disease spectrum has trended toward nonsignificant associations. Nonetheless, a histologic inflammatory gradient has been observed in BE, defined by higher levels of IL1 β and IL8, and was lowest at the esophageal z-line and progressively higher toward the new squamocolumnar junction of the BE lesion distal from the gastroesophageal junction (GEJ).³⁷ Further studies by Abdel-Latif et al³⁸ observed that IL1 β expression increased progressively, coincidental with NF- κ B transcriptional activation, from esophagitis through BE-EAC. Interestingly, reflux-mediated induction of IL1 β using in vitro models was higher in EAC cells than cells of squamous origins, most likely owing to differences in NF- κ B status.^{39,40} Evidence from murine modeling suggests that overexpression of IL1 β in mouse forestomach may lead to IL6-dependent intestine-like metaplastic pathologies and tumor-like growths in the junction between the esophagus and the forestomach, which in mice sits midway through the stomach, rather than the higher esophageal cardia junction anatomic location in *Homo sapiens*, canine, and porcine.⁴¹⁻⁴³ Gastric-specific expression of IL1 β in C57BL/6J transgenic mice has long been known to induce gastric dysplasia with marked inflammation and early infiltration of myeloid-derived suppressor cells (MDSCs).³⁴ High levels of IL1 β are a powerful mediator of acid-suppressive gastritis, with greater potency on acid secretion than proton pump inhibitors (PPIs) such as omeprazole.^{37,41} Fu et al²¹ recently showed that CD44⁺ cells negative for leucine rich repeat containing G protein-coupled receptor 5 (LGR5) at the murine GEJ were more susceptible to oncogenic transformation to GEJ carcinoma, by inactivation of tumor-suppressor genes *Trp53* and *Rb1*, than their antral cousins. Similarly, KRAS^{G12D}- and p53mut-dependent transformation of KRT15⁺ cells at the murine squamocolumnar junction (SCJ) was higher than observed in the regions distal from the SCJ.²² This transitional zone may hold precursor cells, at least in mice, that may be predisposed to oncogenic transformations through either inflammatory mechanisms or somatic variation. It is unclear, however, whether these models more appropriately mirror gastric-associated metaplastic transformation linked to gastric cancers rather than the reflux-driven and BE-associated EAC. Nevertheless, these data do suggest a link between localized inflammation and stem cell fate decisions in metaplastic-like transformations of mouse forestomach, but no reports of genomic instability or somatic variations have been reported in these animal models.^{41,42} Recent evidence pointing toward a gastric cardia origin for the BE lesion, however,⁴⁴ suggests that communication between IL1 β released at sites of GERD-wounding in the esophagus and cardia resident stem cell populations, such as Lgr5⁺ CD44⁺ cells, warrants critical analysis. In canine reflux models, the presence of squamous barriers to proximal migration

of columnar epithelium was not capable of inhibiting columnar metaplasia formation.⁴⁵ Thus, further functional and anatomic investigations are needed with appropriate animal or in vitro models for the improved replication of esophageal adenocarcinogenesis.

Death or Regeneration: TNF α

TNF α initially was noted for its cytotoxic effects on tumor cells; however, further studies elucidated both apoptotic and anti-apoptotic functions in the context of inflammatory responses.^{55–58} The secretion of TNF α is provided primarily by macrophages and follicular dendritic reticular cells, mast cells, and lymphocytes.^{55,57–61} The greatest context of TNF α signaling is, to a large extent, provided by its 2 receptors: TNF-receptor (TNFR)1 and TNFR2. Both receptors may facilitate inflammatory activation through transcription factor NF- κ B^{62,63}; however, it is broadly proposed that TNFR2 transmits a cell survival signal through cellular inhibitor of apoptosis protein 1/2 (cIAP1/2) and BCL2 apoptosis regulator like 2 (BCL2L1 or BCL-XL) activation and TNFR1, inducing a wider array of potential outcomes through the formation of receptor-interacting serine/threonine-protein kinase 1-containing complexes that induce cellular apoptosis and necroptosis. The apoptotic arm of TNF signaling is mediated through TNFR1 and the formation of the death inducing signaling complexes.^{56,64–67} The survival component is similarly transmitted through TNFR1, but it involves the ubiquitination-mediated degradation of bound receptor-interacting serine/threonine-protein kinase 1 protein factor^{56,63,64} and the activation of a kinase relay that induces survival-mediating pathways such as c-Jun N-terminal kinases, p38, and the critical inflammatory transcription factor NF- κ B.^{62,63} Subsequently, transcription and secretion of potent proinflammatory cytokines—particularly IL1 β and IL6—occurs in target cells, be they epithelial, stromal, or of immune origin (Figures 1 and 2).^{66,68} Although this response traditionally is associated with the initiation of an inflammatory response against microbial pathogens in activated antigen-presenting cells, TNF α -mediated survival also may be hijacked by cancer cell populations.^{69–71}

The proinflammatory role of TNF α has been implicated in chronic inflammation underlying the BE–high-grade dysplasia (HGD)–EAC progression.^{72,73} Data from nearly 300 EAC patients showed a high level of circulating TNF α and the associated inflammatory C-reactive protein marker is associated significantly with EAC.⁷² In addition, significantly high levels of soluble TNF α -receptor 2, suggested to originate from uncontrolled shedding of classically membrane-bound TNF α -receptor 2 in EAC (Figure 2), was suggested to result in continuous aberrant inflammatory responses. Intriguingly, TNF α expression was higher in tumor biopsy specimens of EAC patients with pre-existing GERD, suggesting an amplified response mediated by the consistent exposure of esophageal tissue to bile reflux during GERD.⁷² The bile acid deoxycholate, present in the reflux of GERD patients, has been observed to induce both apoptosis and COX2-regulated cell survival in EAC cell lines in a similar fashion to TNF α .⁷⁴ This apoptotic stasis

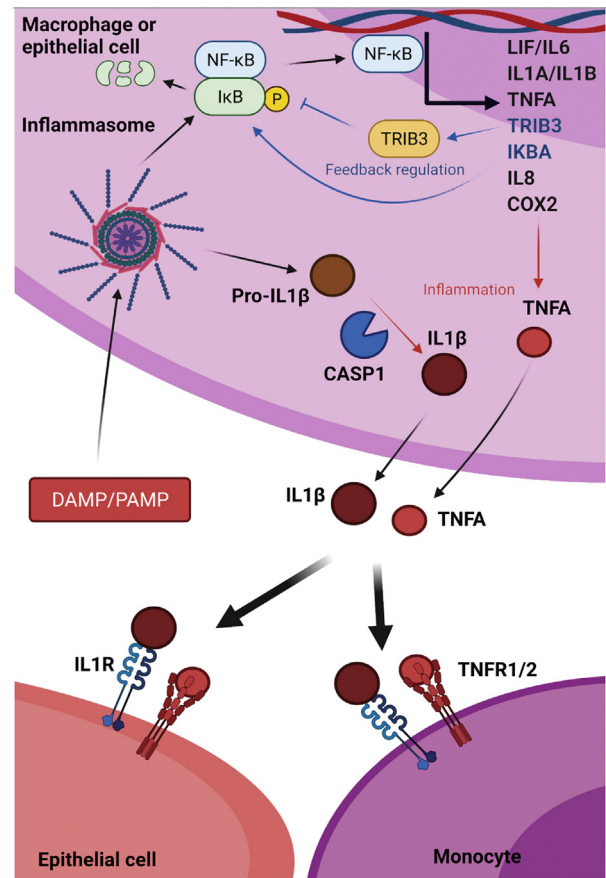


Figure 1. Inflammatory starting line. Damage-associated molecular pattern molecules (DAMP), resulting from GERD tissue damage, induce the formation and activity of the nucleotide-binding oligomerization domain–like receptor pyrin domain containing 3 (NLRP3) inflammasome and cytokine release. Pro-IL1 β is cleaved by the inflammasome and caspase 1 into its active form, followed by release of both IL1 β and TNF α from tissue resident monocytes and macrophages. IL1 β recruits monocytes from the bone marrow— whose arrival and exposure to further IL1 β , TNF α largely through TNFR2-receptor engagement, and GERD constituents—further amplifies the inflammatory response. The presence of TNFR1 and/or TNFR2 receptors dictate the specificity of the response to TNF α exposure and the induction of inflammatory cytokines, such as IL6 and IL8, to further increase TNF α messenger RNA transcription via NF- κ B transcription factor activation. Exposure of esophageal epithelial cells to IL1 β and TNF α results in pathway activations in a cell type and pathology-specific manner. Both exogenous IL1 β and TNF α may initiate phosphorylation-dependent signaling in epithelial cells through the I κ B kinase complex, resulting in phosphorylation and ubiquitination of I κ B α , subsequent release of the NF- κ B transcription factor, and translocation into the nucleus to induce inflammatory cytokine and prostaglandin production (IL8, IL6, LIF, and COX2). IL1 β and TNF α signaling may be suppressed by the initiation of feedback regulators such as the IL1RA-receptor antagonist, I κ B α replenishment, and induction of the noncanonical pseudokinase tribbles homology 3 (TRIB3), which acts to block phosphorylation of I κ B α . Mounting evidence suggests tissue- and cell type-specific responses to bile acid and acid reflux, especially when comparing between normal esophageal and metaplastic tissues.^{26,37–39,46–54} CASP1, Caspase 1; PAMP, Pathogen-associated molecular pattern; TNFA, Tumor necrosis factor alpha.

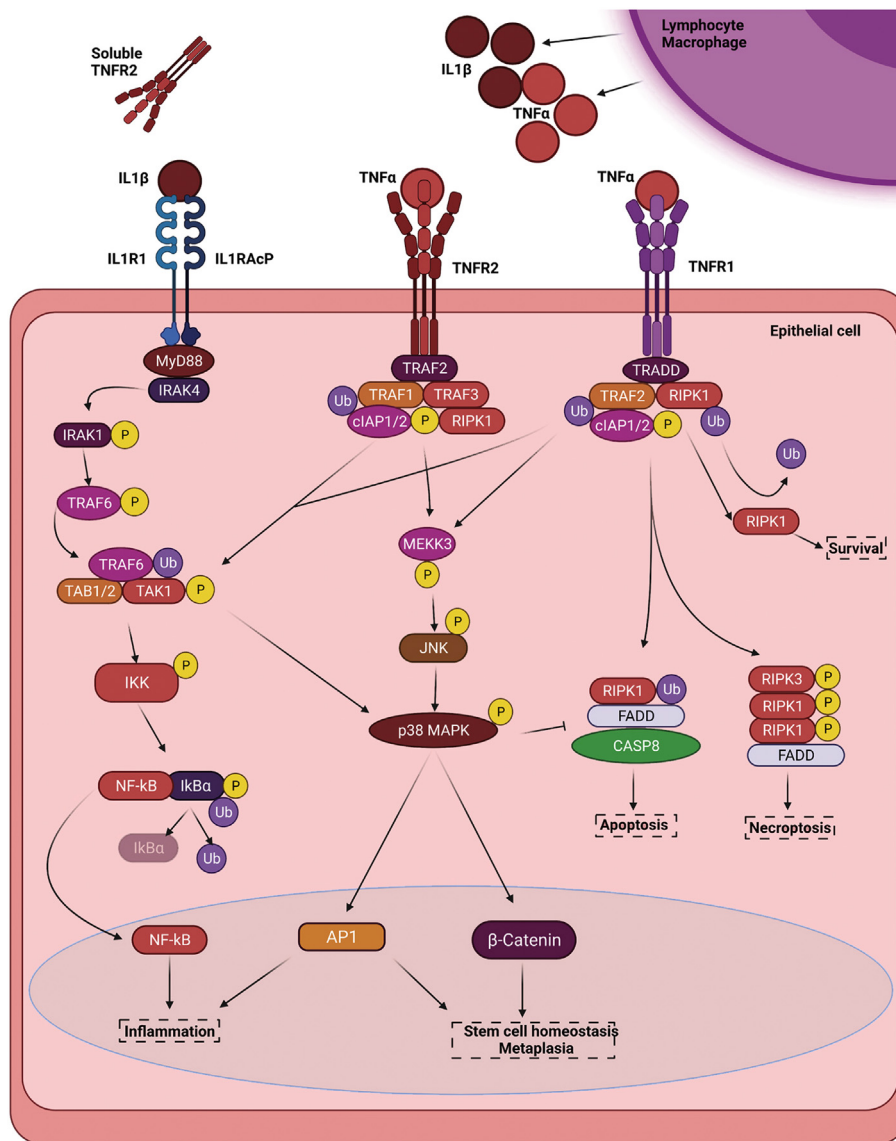


Figure 2. Overlapping and context-specific signaling in response to IL1β and TNFα. Dimerization of IL1R1 with IL1RacP transmits the IL1β signal through the MyD88 adapter protein and activation of an IL1-receptor associated kinase (IRAK) cascade. Subsequent phosphorylation of the E3 ligase TNF-receptor-associated factor 6 (TRAF6) induces the polyubiquitination of the TAB1/2 protein and autophosphorylation of TAK1 in complex. This signalosome is then responsible for the activation of the IκB kinase (IKK) complex comprising of IκBα, IκBβ, and NEMO; the phosphorylation and polyubiquitination of IκBα; the release of NF-κB transcription factor; and the subsequent inflammation. TNFα may transmit through TNFR1 and TNFR2 receptors expressed on a wide variety of cells together or independently. The TNFR2 receptor is engaged for inflammatory activation but also may regulate β-catenin activity, as observed in EAC, to impact tissue homeostasis and cell survival signaling through anti-apoptotic gene expression. TNFR1 shows a more restricted expression pattern and regulates death mechanisms through apoptosis, necrosis, or survival in response to TNFα. Both receptors use the TRAF family of E3 ligases to polyubiquitinate downstream targets such as cellular inhibitor of apoptosis protein family members (cIAP1/2), IκBα, and receptor-interacting serine/threonine-protein kinase 1 (RIPK1). Activation of inflammatory signaling through NF-κB is mediated through cIAP1/2-to-TAK1 ubiquitination and the IKK complex activation. In the case of TNFR2, survival and stem cell homeostatic signaling is communicated through a c-Jun N-terminal kinase (JNK)-p38 MAPK activation phosphorylation cascade resulting in AP1 and α-catenin transcriptional activation. TNFR1 activation, on the other hand, dictates survival or death responses through the function of the receptor-interacting protein (RIP) kinase family. The ubiquitination or phosphorylation status of the RIPK1 protein appears to be the predominant dictator of cell fate. Interactions between RIPK1, Fas-associated protein with death domain (FADD), and the CASP8 caspase define an apoptotic response, as opposed to phosphorylation of RIPK1 and its complex with RIPK3 and FADD, of which defines a necroptosis response. Mitigating this signaling is the process of ubiquitination and deubiquitylation of the RIPK1 protein by proteins such as cIAP1/2, resulting in a balance between survival and apoptosis. ILRacP, Interleukin 1 Receptor Accessory Protein; MyD88, Myeloid differentiation primary response 88; TAK, TGF-β-activated kinase; TRAF6, Transforming growth factor-β (TGF-β)-activated kinase 6; TAB1/2, TAK-binding protein 1/2; NEMO, NF-κB essential modulator; CASP8, Caspase 8.

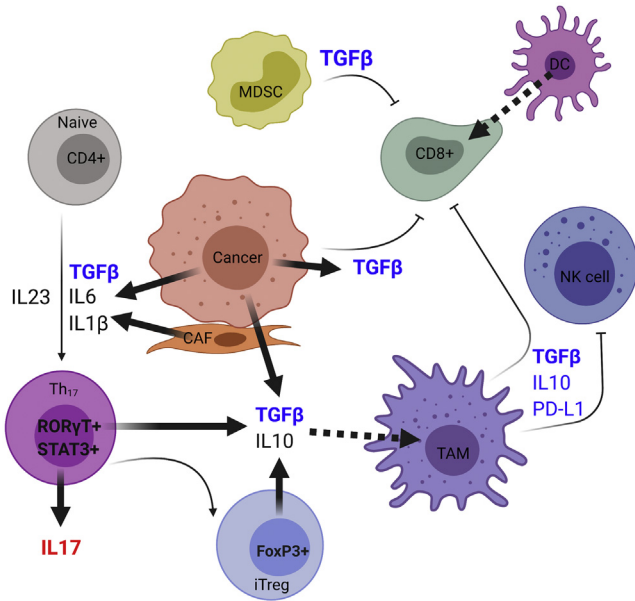


Figure 3. The multiple sources and immune functions of TGF β in cancer. TGF β , produced by cancer cells and tumor-associated immune cells such as MDSCs, plays a prominent role in supporting the differentiation of naive immune cells in response to IL6-mediated STAT activation.^{98–115} Sources of IL6 include epithelial cancer cells, cancer-associated fibroblasts, and, in the case of treatment-naïve esophageal adenocarcinoma cases, ongoing GERD-mediated injury of the surface epithelium.^{49,50,116–120} Together, IL1 β , TGF β , IL23, and IL6 promote peripheral differentiation of naive CD4⁺ T cells into inflammatory IL17-producing ROR γ T⁺ T cells (Th17). Large amounts of immunosuppressive cytokines, particularly IL10 and TGF β , from M2 TAMs and the tumor itself drive ROR γ T inhibition, subsequently switching a predominantly inflammatory Th17 CD4⁺ T-cell population toward a predominantly immunosuppressive inhibitory T-regulatory cell (iTreg) (FoxP3⁺) population.^{99,101,103,107,108,113,121} TAMs, in turn, through the action of TGF β and PD-L1, can inhibit the activity-infiltrating cytotoxic CD8⁺ T cells and cancer surveillant NK cells. MDSCs also may provide immunosuppressive activities through TGF β production. DC, dendritic cell; ROR γ T, retinoid-related orphan receptor gamma t; PD-L1, programmed death-1 ligand 1 (PD-L1).

or resistance is likely to further impact TNF α -mediated signaling events during EAC pathogenesis. Indeed, stage-specific induction of IL8 and epithelial-to-mesenchymal transition in EAC cell lines to TNF α treatment have been noted recently.⁷⁵ Comparatively, in other studies, conditioned media from esophageal cancer cell lines was capable of reducing TNF α levels of lipopolysaccharide-activated dendritic cells.⁷⁶ This intriguing juxtaposition requires further attention but also necessitates the significant widening of the commercially available EAC cell lines—which currently consists largely of SKGT4, OE33, and FLO1 for the most part—or the transition into the use

of cancer-associated organoid culture systems. The wingless WNT/ β -catenin pathway is an essential component of intestinal homeostasis and the intestine-like phenotype and expression pattern underlying BE and EAC development. In familial adenomatous polyposis, mutational loss of function of the *APC* gene results in overactive β -catenin signaling and subsequent promotion of cancer stemness in colonic epithelial cells.^{77–79} TNF α levels become progressively higher from metaplasia-dysplasia-carcinoma and, in early data, were observed to up-regulate the *c-myc* oncogene through β -catenin action independent from NF- κ B in esophageal cells (Figure 2).⁴⁶ This evidence suggests that, although TNF α plays a significant role in the maintenance of highly inflammatory microenvironments through NF- κ B and its downstream targets, it also directly connects the inflammatory milieu with possible mediators of dysplastic and tumorigenic transformation.^{46,69,74} Since these early findings, however, no further connections between TNF α and intestine-like commitment of tissue resident stem cells of the lower esophagus have been noted.

All Roads Lead to Rome: NF- κ B

The NF- κ B transcription factor is central to the proinflammatory response, cellular proliferation, and metastasis associated with the development and progression of various human cancers, including EAC.^{80,81} The canonical pathway involves NF- κ B inducing kinase-mediated transmission of receptor signaling to elicit the degradation of the inhibitor of nuclear factor kappa B (*I* κ B) inhibitory protein via the *I* κ B kinase complex, ultimately resulting in the release of the NF- κ B p65/p50 heterodimer and higher levels of downstream transcriptional activity.^{82–84} Comparatively, the noncanonical pathway consists of the formation of a tumor necrosis factor receptor-associated factor (TRAF)/cIAP1/2/NF- κ B-inducing kinase complex, subsequent *I* κ B kinase α phosphorylation, and the activation of RELB proto-oncogene, NF- κ B subunit (RelB) heterodimerized with either the p52 or p100 form of nuclear factor kappa B subunit 2 (p52) to form the RelB/p52 or RelB/p100 NF- κ B heterodimers.^{81,85,86} Although metastatic potential, cell proliferation, apoptotic evasion, and acute inflammatory response have been linked to both pathway arms, the noncanonical pathway may have a more important role in prolonged inflammation owing to its delayed activation through CD40 ligand (CD40L), TNF superfamily member 13b (TNSF13B or BAFF), and TNF superfamily member 12 (TNFS12 or TWEAK) ligands, among others.^{84,85,87–89} Canonical activation on the other hand can be induced by classic ligand-receptor cytokines such as IL1 β , IL8, and TNF α , as defined earlier^{90–93} (Figure 3). In addition, NF- κ B is capable of transcribing pro-IL1 β , IL8, and TNF α .^{94–97}

The inflammatory response to reflux stress can be prompted through numerous means, not all of which may be through the caustic damage initiated by gastric bile acids and subsequent innate responses. Nonapoptotic concentrations of bile acids and minor changes in pH have been

shown to result in the activation of NF- κ B through the degradation of inhibitory proteins such as I κ B α in esophageal epithelial cells, subsequently inducing the expression and release of cytokines, such as IL8 and IL6, and the prostaglandin-synthesizing enzyme COX2^{38,39,47,80} (Figure 1). In vitro experiments by Abdel-Latif et al.³⁸ in 2004 showed increased activity of NF- κ B in esophageal cell lines exposed to a solution of pH 4, mirroring reflux events, in addition to showing significantly lower levels of the NF- κ B-suppressive I κ B α protein in EAC tumor samples when compared to normal esophageal tissue. This suggests that in EAC tumor cells, the increased levels of active NF- κ B may be owed to the increased phosphorylation and subsequent degradation of I κ B α in response to prolonged acid damage. This is responsible for the observed increase in IL8 levels throughout the BE–EAC sequence. Thus, at some critical point in the GERD–BE–EAC sequence, constitutive activation of NF- κ B and inflammatory proteins, such as IL8, occurs. Subsequent follow-up studies uncovered that the higher level of IL8, IL6, and COX2 expression observed in BE and EAC biopsy specimens was mediated by lower expression of the noncanonical NF- κ B regulator tribbles homology 3³⁹ (Figure 1). In this study, the mechanism underlying these altered inflammatory responses between cells at either end of the EAC sequence was uncovered using exposure to exogenous deoxycholic acid, a component of bile refluxate. Thus, differing inflammatory IL8 and COX2 signaling observed throughout the squamous BE–EAC sequence may result from cell type-specific losses of tribbles homology 3 expression and function, among other avenues. In summary, hyperactivation of NF- κ B through both constitutive proinflammatory signaling and/or loss of negative regulation along the GERD–BE–EAC sequence are common routes to providing a prometaplastic and protumorigenic niche; however, the evidence connecting early activation of NF- κ B with the occurrence of genetic instability during dysplasia is less clear.

Signs of its Passing: Increased Prostaglandins

Prostaglandins are lipid autacoids whose levels increase at sites of wounding or infection and may have both inflammatory and suppressive roles mediated by G-protein-coupled receptor binding in early and late phases of inflammation. Notably, prostaglandins are involved in some of the more visible signs of inflammation such as redness, swelling, and pain. The COX1 and COX2 enzymes together with tissue-specific isomerases convert arachidonic acid into a variety of prostanoids. Although the COX2 synthase, traditionally under the control of NF- κ B, mediates the synthesis of many prostaglandin isoforms from arachidonic acid, the most prevalently studied in the context of adenocarcinogenesis is prostaglandin E2 (PGE2).^{48,122,123} In early work, both low pH and bile acid exposure was shown to result in COX2 (prostaglandin-endoperoxide synthase 2 *PTGS2*) gene expression in a wide variety of esophageal cell

lines.^{39,47,124} Interestingly, Jimenez et al.⁴⁸ found that the EP2 and EP4 PGE2-receptor subtypes were overexpressed in BE and EAC cell lines, respectively. In addition, they showed that bile acid exposure increased both COX2 and several PGE2-receptor subtypes, suggesting that autocrine signaling of PGE2 may play a role in the BE to EAC sequence.⁴⁸ More recently, PGE2 production by BE tissues were found to be significantly higher than non-erosive reflux disease tissues exposed to reflux.¹²⁵ Although differential functions of PGE2 in BE and EAC mediated by different receptor expression is not well detailed, there are several mechanisms by which PGE2 may promote inflammation and mediate the formation of a protumorigenic microenvironment. Recent work by Moon et al.^{22,80} neatly showed the ability of PPI treatment to suppress Ras/p53-driven tumor formation in the Keratin KRT15⁺ population at the murine SCJ and additionally showed, using both murine and organoid experimentation, that this may be mediated through affects on COX2 activity.

A Torrential Rain of Cytokines: LIF, IL6, and the STAT3 Axis

Studies have suggested that intrinsic, aberrant STAT3 activation upon BE–HGD–EAC development increases the ability of these cells to evade apoptosis in response to low pH and/or bile acid-rich environments.^{40,116,126} IL6, a pleiotropic inflammatory and lineage-specifying cytokine, is produced by both epithelial, stromal, and immune cells alike.^{127–130} In the classic pathway, IL6 binds to membrane-bound IL6R, initiating a conformational change that complexes with 2 each of IL6, IL6R, and IL6-receptor subunit- β (gp130).¹³¹ LIF, a cytokine sharing the gp130 receptor subunit with the IL6 family, is expressed in a wide variety of tissue types with functions linked to reproduction, stem cell growth, and embryonic development.^{132–134} Both IL6 and LIF signaling lead to nonreceptor tyrosine kinase Janus kinase 3 (JAK3) activation and subsequent dimerization of the STAT3 transcription factor via phosphorylation.¹³¹ Nuclear translocation of phospho-STAT3 allows promoter binding at target genes such as *VEGF-A*, *TGF β* , *Bcl-xL*, and *Mcl-1*.¹¹⁷ The consequences of STAT3-mediated transcription include inflammation/immune response, cell survival, metastasis, and angiogenesis, among others, with variations in function between epithelial and immune cells.^{40,49,116–118,126,131,135}

Recent RNA sequencing expression studies from a variety of patient-derived BE, HGD, and EAC tissues has shown a significantly large increase in tissue IL6 expression along the adenocarcinogenesis sequence.⁵⁰ Hyperactivation of IL6/STAT3 signaling in both dysplastic and EAC tissues has been linked to JAK–STAT3-mediated promotion of cancer cell survival in response to bile acid treatment through BCL-XL¹¹⁶ and MCL1¹²⁶ levels, which may also be p53-dependent; however, induction of STAT3 signaling also has been observed to be mediated additionally through an epidermal growth factor receptor and Apurinic/

aprimidinic endonuclease-1 (APE1)-mediated axis in a redox-dependent manner.¹³⁶ Although largely a model of murine gastric cancer, the forced overexpression of IL1 β in the murine midstomach was observed to promote dysplasia-like lesions through an IL6-dependent mechanism in the stomach.⁴¹ With recent data pointing toward cardia resident origin of BE stem cells, it may be time to further develop and independently verify this complicated murine cancer model. Divergent from IL6, GERD-associated bile acid-mediated induction of LIF has been observed to be significantly higher in squamous esophageal cells than EAC cell lines,³⁹ with a later study confirming significantly higher levels of constitutive LIF messenger RNA expression in HGD/EAC samples compared with healthy esophageal tissues via immunohistochemistry.⁴⁰ In this study, a chronological order for autocrine IL6-STAT3 activation through LIF secretion in EAC cells was determined.⁴⁰ LIF-mediated STAT3 is long known to induce the embryonic stem cell self-renewal transcription factor SRY-box transcription factor 2 (SOX2), an important tissue patterning transcription factor altered during the BE-HGD-EAC sequence.^{51,137} Recent chromatin immunoprecipitation sequencing analysis of several EAC (n = 20) cells, both of patients (n = 11) and cell lines (n = 9), and ESCC (n = 6) cells showed EAC-specific enrichment of cytokine signaling-related enhancers.¹³⁸ LIF was the most highly enriched EAC-specific cytokine, and its expression is highly dependent on the activity of 4 key master transcription factors that bind to EAC-specific superenhancers: E74 like ETS transcription factor 3 (ELF3), Kruppel like factor 5 (KLF5), ETS homologous factor (EHF), and GATA binding protein 6 (GATA6).¹³⁸ Interestingly, of these 4 EAC-specific master TFs, GATA6 has been implicated as a key driver of the metaplastic transformation of esophageal tissue and survival of oncogenic BE populations,^{52,98,139-142} with somatic variation at the GATA6 locus observed in a significant number of EAC cases according to the cancer genome atlas (TCGA) data. In addition, messenger RNA expression of GATA6 is bile acid-inducible in dysplastic BE and EAC cells, in addition to increased expression along the BE-HGD-EAC progression.⁵² Therefore, the complex interplay between LIF, BE stem cells, and GATA factors in EAC development requires further investigation and may provide a promising target in immunotherapies against EAC.

A higher M2/M1 ratio of tumor-associated macrophages (TAMs) is associated with increased EAC tumor metastatic potential, possibly owing to the immunosuppressive capacities of M2-type TAMs, which drive tumor immune escape.¹⁴³ In vitro studies have shown that both LIF and IL6 are required for the differentiation of peripheral blood mononuclear cells toward an M2 TAM phenotype in studies involving an ovarian carcinoma tumor microenvironment (TME).¹⁴⁴ Thus, these M2-TAM-promoting cytokines may have potential synergistic actions in the development of EAC and its tumor-immune landscape. Further studies of LIF expression and its relationship to the EAC TME are warranted to fully elucidate LIF's role in the aberrant IL6/STAT3 epithelial axis, in addition to its influence on the immunologic component of the GERD-BE-EAC sequence. A

hypothetical chronological order of events in BE-EAC adenocarcinogenesis mediated by LIF may be as follows: LIF secreted by esophageal squamous and/or inflammatory cells in response to GERD, BE cells become LIF-dependent, GATA6-enriched dysplastic BE cells secrete high levels of LIF, LIF promotes the selection of M2 TAMs, M2 TAMs express high levels of immunosuppressive factors, and dysplastic BE cells progress to prometastatic EAC phenotypes as they evade immunosurveillance. The maintenance of a knife's-edge balance between inflammatory and immunosuppressive factors in the TME through IL6/STAT3 signaling, and pathologic cell-specific responses to GERD components, may further promote the expression of anti-apoptotic proteins that encourage malignant transformation and clonal selection during EAC development.^{116,126}

Controlling the Immune Narrative: Transforming Growth Factor- β Family

The cytokine TGF β is a pleiotropic regulator involved in epithelial and immune homeostasis; immune tolerance; and context-dependent effects on cellular differentiation, development, and tissue homeostasis.^{145,146} The wider TGF β protein family consists of 2 main branches with TGF β 1-3, activin, myostatin, nodal, growth differentiation factor (GDF)-1 and GDF-3, inhibin, and Lefty-1 and Lefty-2 on one arm¹⁴⁶⁻¹⁴⁸; and a second bone morphogenetic protein family branch containing a variety of bone morphogenetic proteins, GDF-5, GDF-6, GDF-7, and anti-Mullerian hormone (AMH/MIS).¹⁴⁶⁻¹⁴⁸ A third and distant branch of the TGF β superfamily consists of GDF-15, which has been implicated as a driver of metastasis in several cancers, and, more recently, metabolic homeostasis resulting from emerging evidence suggestive of binding to glial-derived neurotrophic factor family receptor α -like.^{99,100,149-152} Dysregulation of TGF β signaling plays a key role in the development of cancer by promoting both epithelial tumor phenotypes and immunotolerance. Immunologically, TGF β can promote or suppress the differentiation of various immune cell effectors that are dependent on the modulation of dynamic environmental cofactors such as IL6 cytokine levels.^{147,101,153,154}

At steady-state, TGF β maintains the homeostasis of peripheral CD4+ and CD8+ T-cell levels through a canonical signaling pathway. This is dependent on small mothers against decapentaplegic family member 3 (SMAD3), a receptor-regulated SMAD that mediates the suppression of proinflammatory IL2.^{147,101,153,154} The secretion of proinflammatory cytokines, such as IL6 and IL1 β , from activated macrophages, dendritic cells, and neutrophils, bring CD4+ and CD8+ T cells out of a steady-state and toward mature effector phenotypes that now may be directed by localized TGF β .^{147,153} (Figure 3). For example, TGF β and high IL6 promotes autocrine production of IL23 from naive CD4+ T cells in a synergistic and concentration-dependent manner, and this IL23 autocrine signaling has been defined to underlie the formation of proinflammatory type 17 T-helper cell (Th17) lineages.^{102,103,147,153} (Figure 3). In a cancer context, TGF β can suppress the cytotoxic action of cancer surveillant natural killer (NK) cells and CD8+ effector T

cells (Figure 3). This mechanism can be explained via the suppressive action of the TGF β -activated SMAD-ATF1 repressor complex on perforin, granzyme A/B, Fas-ligand, and interferon γ (IFN- γ).¹⁰⁴ In addition, at the post-translational level, Jun et al¹⁵⁵ showed a degranulation response prevention in NK cells exposed to TGF β , preventing the exocytosis of IFN- γ -containing granules required for proper NK-mediated cytotoxic responses. Over the past 30 years, several studies on EAC patient cohorts consistently have shown a significant correlation between patient mortality and the lack of tumor-infiltrating CD8+ T cells and NK cells.¹⁵⁶⁻¹⁵⁸ Recent evidence has shown that this may be owed to the promotion of immunotolerance by an enrichment of inhibitory T-regulatory cells in more advanced tumors, and the possible mechanism by which inhibitory T-regulatory cells promote immunotolerance is through programmed cell death protein 1-mediated apoptosis of CD8+ T cells (Figure 3).¹⁰⁵ NK cell levels in esophagogastric tumors, including EAC, may be variable and associated with loss of heterozygosity at chromosome 4 and what has become known as a cold immune phenotype defined by low CD8 and IFN- γ -producing cells, supporting the hypothesis of a TGF β -mediated role in poor outcomes through immunologic means in addition to its epithelial impacts.¹⁵⁹ In this fashion, interactions between MDSCs and TGF β is implicated in supporting tumor growth through immunosuppressive functions.^{34,160} Prolonged inflammatory states, such as those found in BE in the esophagus or inflammatory bowel disease/ulcerative colitis in the colon, allow for the selection of epithelial cells that are more resistant to the growth-suppressive, pro-apoptotic effects of canonical TGF β signaling.^{106,161,162} This protumorigenic selection, combined with the suppression of cytotoxic NK and CD8+ effector T cells induced by TGF β and chemoattraction of MDSCs, increases the probability of tumorigenesis.^{102,103,106-108,161-164} Developing immunotherapies that promote tumor infiltration by NK and CD8+ T cells, therefore, may be a key future therapeutic strategy.

The Ties That Bind: Complement Components

Complement component 1q (C1q) is a soluble protein complex acting as a key bridge between innate and adaptive immune responses.¹⁶⁵⁻¹⁶⁷ C1q is secreted primarily by monocyte-lineage cells such as macrophages, and, at steady-state, circulating blood C1q is found at relatively low levels.¹⁶⁵⁻¹⁶⁸ During infection and tissue damage, C1q binds to the fragment crystallizable (Fc) region of circulating IgG or IgM antibodies, apoptotic cells, bacterial surfaces, and ligand-bound C-reactive protein.¹⁶⁵⁻¹⁶⁷ This allows for the aggregation of antigen-antibody-C1q complexes to form.^{165,166} This process, known as opsonization, allows for increased phagocytic efficiency of cells such as macrophages via the classic complement cascade, which ends with the formation of a membrane attack complex that initiates target cell lysis.¹⁶⁵⁻¹⁶⁷ C1q immunoreactivity is increased significantly in the aging brain, and serum levels remain stable up until 40 years of age, after which C1q levels

increase progressively with age,¹⁶⁹ intriguingly tracking the age-related propensity for cancer development.

In the cancer TME, the complement system and its constituent components are proposed to be engaged in the antibody-mediated killing of targeted cancer cells, support chronic inflammatory states, or indeed hamper antitumor responses. Furthermore, some complement components have been observed to support tumor neoangiogenesis. Clinical data from a recent cohort of 134 colon, lung, lymphatic, and blood cancer patients, with circulating C1q levels higher than the 95th percentile, indicated a strong correlation between enriched blood C1q and post-treatment failure.¹⁷⁰ Increased tumoral C1q recently has been associated with progressive BE-to-EAC adenocarcinogenesis and tumor survival.⁴⁰ In this study, overexpression of C1q subcomponent A chain transcript and protein were found in both BE and EAC patient samples and cell lines. Small interfering RNA-mediated silencing of C1q subcomponent A chain transcription resulted in reduced EAC cell growth through a complement-independent pathway. Supportively, C1q silencing-mediated suppression of in vitro tumor cell growth was restored upon treatment with native human C1q protein. In addition, LIF was proposed as a key regulator in the induction of C1q transcript in EAC cells, suggesting that C1q, in addition to JAK/STAT and spleen tyrosine kinase (SYK)/protein kinase B (PKB or AKT) activation, may be a possible downstream effector of LIF.⁴⁰ Thus, as proposed and shown by Duggan et al⁴⁰, SYK inhibitors, such as fostamatinib, that conventionally target B-cell-driven autoimmune conditions, were capable of inhibiting both STAT and SYK action in EAC cells that reduced their growth. Therefore, fostamatinib may provide a promising method of targeted-treatment against C1q/LIF-enriched EAC tumors.¹⁷¹ Interestingly, the Bruton's tyrosine kinase inhibitor ibrutinib, indicated for B-cell lymphoma and leukemia, is a promising therapeutic against MYC proto-oncogene, bHLH transcription factor (MYC)-amplified EAC tumors, thus further supporting the narrative of innate immune involvement in EAC development.¹⁷² More recently, Wnt/ β -catenin signaling, a critical component of the underlying metaplastic process leading to EAC and a direct regulator of MYC expression, has been shown to be modulated by exposure to C1q in murine aging studies.¹⁷³ This mechanism may explain the hyperactivated Wnt signaling observed in geriatric-associated cancers driven through oncogenic β -catenin activity and high C1q levels.¹⁷³⁻¹⁷⁵ Thus, the cellular and protein facilitators of innate immunity may have vital roles in EAC development, given the age profile of EAC patients and the alterations in Wnt/ β -catenin signaling in this cancer type.

The Bugle's Call: Immune Cell Dynamics in GERD-BE-EAC Transition

Characterizing the population of immune cells infiltrating the esophageal epithelium along BE-EAC progression may provide insight into the aims, resolution, and processing of the local inflammatory or immune response to environmental stressors such as GERD. Studies on the identity of

immune cell infiltration in response to GERD and upon BE development are surprisingly scarce; however, they provide some initial insight to the cellular immune microenvironment that potentially shapes and perhaps drives the malignant transition of esophageal epithelium. Current evidence points tentatively towards dynamic populational shifts during this carcinogenic timeline, beginning as a predominantly proinflammatory CD4+ T-cell infiltrate that shifts to one that may be immunosuppressive in late-stage EAC. The proportion of Th1 and Th2 signatures have been used primarily to represent shifts between distinct tissue-immune environments, because Th1 cells primarily secrete IL2 and IFN- γ , whereas Th2 cells secrete IL4 and IL10.^{176,177} Fitzgerald et al¹⁷⁸ first investigated Th1/Th2 signatures in esophageal biopsy specimens from normal esophagus, esophagitis, and BE lesions. Cytokines IL1 β , IL8, and IFN- γ are indicative (at the time of publication) of a Th1 CD4+ signature; however, in later years, many novel Th subsets have since been investigated but unexplored in the esophageal context. In contrast, BE samples were enriched significantly in IL4 expression compared with esophagitis samples, suggesting a more predominant Th2 signature. In the context of epithelial cell metaplastic transformation, a previous study showed that a predominant Th2 cytokine signature, particularly IL4, has been shown to contribute to the down-regulation of esophageal squamous cell markers and increase in columnar cell signature¹⁷⁹; however, there now is considerable doubt that BE emerges from a squamous-associated stem cell type. A later study by Kavanagh et al,¹⁸⁰ using flow cytometry, observed significantly lower overall numbers of CD4+ cells in EAC tissues compared with both esophagitis and BE samples, suggesting that, over time, the presence of activated T cells within tumors is diminished, most likely owing to an immunosuppressive influence. Similar to previous studies, a significantly higher level of IL4-expressing CD4+ cells were observed in BE tissues, but no other markers of a Th2 response reached significance in this study.¹⁸⁰ Significantly higher production of proinflammatory IL6, IL1 β , and granulocyte-macrophage colony-stimulating factor (GM-CSF) than IL10 was observed in ex vivo cultures of BE tissues when compared to normal esophagus with no alterations in IL4 levels. This may suggest that the Th2-type impression of BE is not fully defined yet and requires significantly higher sample numbers and the use of a wider array of multiparametric fluorescence-activated cell sorting (FACS) assays or immune-staining panels. Intriguingly, a similar comparative analysis of the immune cell composition between BE and duodenal tissues found a striking similarity that the investigators suggested was owed to the intestine-like homing signals of BE tissues.¹⁸¹ More recent evidence from Lagisetty et al⁵⁰ showed a similar, but more nuanced, trend in immune infiltrate population shifts during the progression from BE, low-grade dysplasia, HGD, and EAC using RNA sequencing and xCell deconvolution analysis (<https://xcell.ucsf.edu/>). From their analysis, both Th1 and Th2 signatures were significantly greater in EAC vs BE samples; however, unlike Kavanagh et al,¹⁸⁰ they did not find a significant increase in overall CD4+ infiltration when

comparing BE with EAC, only between low-grade dysplasia and HGD.^{50,180} In addition, there was a significant and step-wise increase in the pro-B-cell signature from BE to EAC. Of the innate immune infiltrate, the presence of eosinophils decreased significantly along BE adenocarcinogenesis while macrophage/monocyte infiltration increased significantly, with a clear M2 signature in EAC compared with HGD.⁵⁰

Although previous studies have shown distinct shifts in immune cell infiltrate composition along BE adenocarcinogenesis, no studies have directly examined if these shifts may be influenced directly by reflux; however, there have been clinical studies examining the relationship between PPIs and eosinophilic esophagitis (EoE) that showed distinct changes in mucosal eosinophil populations post-treatment.^{182,183} The diagnostic criteria for EoE have been updated to reflect the potential co-existence of EoE and GERD, and that GERD may, in fact, influence the infiltration of eosinophils into esophageal tissue. Indeed, clinical studies have shown PPI treatment reduced the histologic grade of EoE patient lesions over time; however, further studies are required to elucidate the direct influence of changes in GERD status on immune cell infiltrate.¹⁸²⁻¹⁸⁴ Recently, Dunbar et al¹⁸⁵ examined the histologic changes after removal of PPI therapy in GERD patients observing a lymphocyte-predominant inflammatory response, basal cell hyperplasia, with no loss of surface cells at 2 weeks. The investigators thus suggested that pathogenesis of reflux esophagitis may be cytokine-mediated rather than the result of chemical injury.

From these studies, it is unclear how both the innate and adaptive immune infiltrate interact to contribute to the overall lesion milieu. Thus, simultaneous cytometric and cytokine paneling studies of higher-powered cohorts are required to determine this relationship and how they affect the overall immune outcome. Comparing normal squamous esophageal cells with BE and EAC, although still pertinent, may not be as important as comparisons with the GEJ, from which it now is suggested that BE emerges. A recent single-cell RNA sequencing and lineage tracing study proposed that BE arises from the gastric cardia, and that EAC arises from undifferentiated BE cell types.⁴⁴ Thus, investigating the local immune infiltrate within normal gastric antrum or duodenum in addition to the esophagus may be more appropriate comparators when investigating changes in immune cell infiltrate along BE adenocarcinogenesis; however, the inflammatory response of, and damage to, the squamous epithelium in response to GERD still may provide immune signaling supportive of the metaplastic process through promotion of aberrant metaplastic stem cell colonization of the ulcer bed.

Final Thoughts and Future Directions

Although we have begun to understand and explain some of the potential origins of BE, the mechanisms through which GERD supports its emergence and its transition to adenocarcinoma remains elusive and yet still is guilty by association. It is likely that some aspects of acid and bile acid signaling play an important role in emergent genomic

instability, but the impact of chronic or recurrent inflammation at the GEJ cannot be supplanted as a likely driver. The wider immunologic contributors to BE and EAC development have yet to be thoroughly defined, such as those provided by immune cells recruited during GERD, BE, and HGD. These aspects can be studied only from an associative context in clinical samples and have not been as tractable in murine models thus far. Therefore, this review limited its scope to cytokine-mediated signaling in the esophagus and its linkage with the emergence of oncogenic and metaplastic transformation from BE. The majority of the research reviewed herein has been performed using a variety of cell line models of esophageal signaling and tended to lean more upon EAC cell lines than that of normal squamous esophageal cell types. Furthermore, if the true origin of BE is from stem cells within the GEJ then the impact of cytokine signaling upon BE-associated differentiation markers in squamous cells becomes less relevant. Inflammatory signaling in the squamous esophagus, however, is still a key component of BE development whether directly or independently through chemotaxis of immune cells to the GEJ and consequent contributions to metaplasia. Regardless, our research community requires novel solutions, model systems, and platforms for exploring the cellular immunity beneath BE and EAC development that is directed by the cytokine milieu and clearly still an important contributor to metaplasia and dysplasia.

Investment in porcine and canine models in addition to the development of novel platforms may be needed to surmount the obstacles between fundamental basic sciences and translational medicine as it is applied to esophageal pathologies that are poorly modeled in the mouse. Older studies modeling metaplasia in the canine showed a more anatomically correct emergence in response to surgically induced reflux.^{7,186,187} These models support the emergence of supposed BE stem cells from the GEJ and obviously are useful for the investigation of inflammatory contributors; however, antibodies and other tools are currently of limited availability for canine and porcine models, and none thus far have been investigated for mutational burden, a critical component of the inherent oncogenic potential of BE. A quicker route to fundamental, human, relevant, and translational discoveries may be garnered from the use of recent organoid technologies capable of mirroring BE and EAC pathologies *ex vivo*. These approaches, based on cell culturing systems associated with regenerative medicine, allow the propagation of cells and glandular structures from a wide array of pathologies and sufficiently represent the clinical and mutational landscape of the original tissues, at least in early cultures. Under some systems, subculturing over time results in the emergence of dominant clones owing to selective pressure, but this may be dependent on the growth supplements and conditions selected. Such systems have been termed *conditional reprogramming*,¹⁸⁸ ground state stem cell propagation,¹⁸⁹ adult tissue-derived organoids,¹⁹⁰ induced pluripotent stem cell (iPSC)-derived organoids,¹⁹¹ spheroids, and air-liquid interface cultures,^{192,193} and have many useful features and detractors dependent on tissue type and

pathology. In the esophageal context, adult-derived organoids of BE and ground state stem cells have shown promise as platforms for the faithful representations of the original tissues but without immune system involvement. Some success has been obtained in maintaining immune cell presence in air-liquid interface cultures of tumor tissues for at least the initial few weeks of culture through the use of IL2-mediated stimulation.^{192,194} Along these lines, a number of lab-on-a-chip platforms that allow co-culture of immune cells flowing through endothelial-coated channels will be useful in combination with patient-derived organoids. Such systems provide interesting models to examine the influence of cytokines on the maintenance of pathologies such as BE and to examine interactions between immune cells with patient-specific pathologies such as BE or EAC. Importantly, single-cell RNA and DNA sequencing of the heterogeneous cell types associated with BE and the resultant cancer may uncover distinct immune profiles or underlying stromal cells with important ramifications that similarly may improve our *ex vivo* modeling. These approaches critically provide preclinical test beds for potential immune-based therapeutics and traditional biologics before further investment in large animal modeling.

In summary, defining the order of this paradoxical inflammatory pathway underlying BE-EAC may not be as critical as studying how, if, or when it reaches resolution and whether this resolution differs between normal esophagus, columnar BE lesions, dysplastic pathologies, and early cancers. To address these questions, we must rely on, and be informed by, the patient population and their continued engagement with research in addition to the continued development of *in vitro* organoid technologies and animal models of the BE-EAC transition.

References

- Huang J, Koulaouzidis A, Marlicz W, Lok V, Chu C, Ngai CH, Zhang L, Chen P, Wang S, Yuan J, Lao XQ, Tse SLA, Xu W, Zheng ZJ, Xie SH, Wong MCS. Global burden, risk factors, and trends of esophageal cancer: an analysis of cancer registries from 48 countries. *Cancers (Basel)* 2021;13:141.
- An J, Gharahkhani P, Law MH, Ong JS, Han X, Olsen CM, Neale RE, Lai J, Vaughan TL, Gockel I, Thieme R, Bohmer AC, Jankowski J, Fitzgerald RC, Schumacher J, Palles C, Beacon, and Me Research T, Whiteman DC, MacGregor S. Gastroesophageal reflux GWAS identifies risk loci that also associate with subsequent severe esophageal diseases. *Nat Commun* 2019;10:4219.
- Buas MF, He Q, Johnson LG, Onstad L, Levine DM, Thrift AP, Gharahkhani P, Palles C, Lagergren J, Fitzgerald RC, Ye W, Caldas C, Bird NC, Shaheen NJ, Bernstein L, Gammon MD, Wu AH, Hardie LJ, Pharoah PD, Liu G, Iyer P, Corley DA, Risch HA, Chow WH, Prenen H, Chegwidden L, Love S, Attwood S, Moayyedi P, MacDonald D, Harrison R, Watson P, Barr H, deCaestecker J, Tomlinson I, Jankowski J, Whiteman DC, MacGregor S, Vaughan TL,

- Madeleine MM. Germline variation in inflammation-related pathways and risk of Barrett's oesophagus and oesophageal adenocarcinoma. *Gut* 2017;66:1739–1747.
4. Su Z, Gay LJ, Strange A, Palles C, Band G, Whiteman DC, Lescai F, Langford C, Nanji M, Edkins S, van der Winkel A, Levine D, Sasieni P, Bellenguez C, Howarth K, Freeman C, Trudgill N, Tucker AT, Pirinen M, Peppelenbosch MP, van der Laan LJ, Kuipers EJ, Drenth JP, Peters WH, Reynolds JV, Kelleher DP, McManus R, Grabsch H, Prenen H, Bisschops R, Krishnadath K, Siersema PD, van Baal JW, Middleton M, Petty R, Gillies R, Burch N, Bhandari P, Paterson S, Edwards C, Penman I, Vaidya K, Ang Y, Murray I, Patel P, Ye W, Mullins P, Wu AH, Bird NC, Dallal H, Shaheen NJ, Murray LJ, Koss K, Bernstein L, Romero Y, Hardie LJ, Zhang R, Winter H, Corley DA, Panter S, Risch HA, Reid BJ, Sargeant I, Gammon MD, Smart H, Dhar A, McMurtry H, Ali H, Liu G, Casson AG, Chow WH, Rutter M, Tawil A, Morris D, Nwokolo C, Isaacs P, Rodgers C, Ragnunath K, MacDonald C, Haigh C, Monk D, Davies G, Wajed S, Johnston D, Gibbons M, Cullen S, Church N, Langley R, Griffin M, Alderson D, Deloukas P, Hunt SE, Gray E, Dronov S, Potter SC, Tashakkori-Ghanbaria A, Anderson M, Brooks C, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Duncanson A, Markus HS, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood N, Trynka G, Wijmenga C, Cazier JB, Atherfold P, Nicholson AM, Gellatly NL, Glancy D, Cooper SC, Cunningham D, Lind T, Hapeshi J, Ferry D, Rathbone B, Brown J, Love S, Attwood S, MacGregor S, Watson P, Sanders S, Ek W, Harrison RF, Moayyedi P, de Caestecker J, Barr H, Stupka E, Vaughan TL, Peltonen L, Spencer CC, Tomlinson I, Donnelly P, Jankowski JA. Esophageal Adenocarcinoma Genetics C, Wellcome Trust Case Control C. Common variants at the MHC locus and at chromosome 16q24.1 predispose to Barrett's esophagus. *Nat Genet* 2012;44:1131–1136.
 5. Zheng J, Erzurumluoglu AM, Elsworth BL, Kemp JP, Howe L, Haycock PC, Hemani G, Tansey K, Laurin C, Early Genetics Lifecourse Epidemiology (EAGLE) Eczema Consortium, Pourcain BS, Warrington NM, Finucane HK, Price AL, Bulik-Sullivan BK, Anttila V, Paternoster L, Gaunt TR, Evans DM, Neale BM. LD Hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. *Bioinformatics* 2017;33:272–279.
 6. Boch JA, Shields HM, Antonioli DA, Zwas F, Sawhney RA, Trier JS. Distribution of cytokeratin markers in Barrett's specialized columnar epithelium. *Gastroenterology* 1997;112:760–765.
 7. Bremner CG, Lynch VP, Ellis FH Jr. Barrett's esophagus: congenital or acquired? An experimental study of esophageal mucosal regeneration in the dog. *Surgery* 1970;68:209–216.
 8. Feurle GE, Helmstaedter V, Buehring A, Bettendorf U, Eckardt VF. Distinct immunohistochemical findings in columnar epithelium of esophageal inlet patch and of Barrett's esophagus. *Dig Dis Sci* 1990;35:86–92.
 9. Garman KS. Origin of Barrett's epithelium: esophageal submucosal glands. *Cell Mol Gastroenterol Hepatol* 2017;4:153–156.
 10. Hutchinson L, Stenstrom B, Chen D, Piperdi B, Levey S, Lyle S, Wang TC, Houghton J. Human Barrett's adenocarcinoma of the esophagus, associated myofibroblasts, and endothelium can arise from bone marrow-derived cells after allogeneic stem cell transplant. *Stem Cells Dev* 2011;20:11–17.
 11. Jankowski JA, Harrison RF, Perry I, Balkwill F, Tselepis C. Barrett's metaplasia. *Lancet* 2000;356:2079–2085.
 12. Jiang M, Li H, Zhang Y, Yang Y, Lu R, Liu K, Lin S, Lan X, Wang H, Wu H, Zhu J, Zhou Z, Xu J, Lee DK, Zhang L, Lee YC, Yuan J, Abrams JA, Wang TC, Sepulveda AR, Wu Q, Chen H, Sun X, She J, Chen X, Que J. Transitional basal cells at the squamous-columnar junction generate Barrett's esophagus. *Nature* 2017;550:529–533.
 13. Lavery DL, Nicholson AM, Poulosom R, Jeffery R, Hussain A, Gay LJ, Jankowski JA, Zeki SS, Barr H, Harrison R, Going J, Kadirkamanathan S, Davis P, Underwood T, Novelli MR, Rodriguez-Justo M, Shepherd N, Jansen M, Wright NA, McDonald SA. The stem cell organisation, and the proliferative and gene expression profile of Barrett's epithelium, replicates pyloric-type gastric glands. *Gut* 2014;63:1854–1863.
 14. Leedham SJ, Preston SL, McDonald SA, Elia G, Bhandari P, Poller D, Harrison R, Novelli MR, Jankowski JA, Wright NA. Individual crypt genetic heterogeneity and the origin of metaplastic glandular epithelium in human Barrett's esophagus. *Gut* 2008;57:1041–1048.
 15. Nicholson AM, Graham TA, Simpson A, Humphries A, Burch N, Rodriguez-Justo M, Novelli M, Harrison R, Wright NA, McDonald SA, Jankowski JA. Barrett's metaplasia glands are clonal, contain multiple stem cells and share a common squamous progenitor. *Gut* 2012;61:1380–1389.
 16. Que J, Garman KS, Souza RF, Spechler SJ. Pathogenesis and cells of origin of Barrett's esophagus. *Gastroenterology* 2019;157:349–364 e1.
 17. Schreiber DS, Apstein M, Hermos JA. Paneth cells in Barrett's esophagus. *Gastroenterology* 1978;74:1302–1304.
 18. Starnes VA, Adkins RB, Ballinger JF, Sawyers JL. Barrett's esophagus. A surgical entity. *Arch Surg* 1984;119:563–567.
 19. Wang X, Ouyang H, Yamamoto Y, Kumar PA, Wei TS, Dagher R, Vincent M, Lu X, Bellizzi AM, Ho KY, Crum CP, Xian W, McKeon F. Residual embryonic cells as precursors of a Barrett's-like metaplasia. *Cell* 2011;145:1023–1035.
 20. Xian W, Ho KY, Crum CP, McKeon F. Cellular origin of Barrett's esophagus: controversy and therapeutic implications. *Gastroenterology* 2012;142:1424–1430.
 21. Fu DJ, Wang L, Chouairi FK, Rose IM, Abetov DA, Miller AD, Yamulla RJ, Schimenti JC, Flesken-Nikitin A, Nikitin AY. Gastric squamous-columnar junction contains a large pool of cancer-prone immature osteopontin responsive Lgr5(-)CD44(+) cells. *Nat Commun* 2020;11:84.

22. Moon H, Zhu J, Donahue LR, Choi E, White AC. Krt5(+)/Krt15(+) foregut basal progenitors give rise to cyclooxygenase-2-dependent tumours in response to gastric acid stress. *Nat Commun* 2019;10:2225.
23. Gaddam S, Singh M, Balasubramanian G, Thota P, Gupta N, Wani S, Higbee AD, Mathur SC, Horwhat JD, Rastogi A, Young PE, Cash BD, Bansal A, Vargo JJ, Falk GW, Lieberman DA, Sampliner RE, Sharma P. Persistence of nondysplastic Barrett's esophagus identifies patients at lower risk for esophageal adenocarcinoma: results from a large multicenter cohort. *Gastroenterology* 2013;145:548–553 e1.
24. Stachler MD, Camarda ND, Deitrick C, Kim A, Agoston AT, Odze RD, Hornick JL, Nag A, Thoner AR, Ducar M, Noffsinger A, Lash RH, Redston M, Carter SL, Davison JM, Bass AJ. Detection of mutations in Barrett's esophagus before progression to high-grade dysplasia or adenocarcinoma. *Gastroenterology* 2018;155:156–167.
25. Weaver JM, Ross-Innes CS, Shannon N, Lynch AG, Forshe T, Barbera M, Murtaza M, Ong CJ, Lao-Sirieix P, Dunning MJ, Smith L, Smith ML, Anderson CL, Carvalho B, O'Donovan M, Underwood TJ, May AP, Grehan N, Hardwick R, Davies J, Oloumi A, Aparicio S, Caldas C, Eldridge MD, Edwards PAW, Rosenfeld N, Tavaré S, Fitzgerald RC; OCCAMS Consortium. Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. *Nat Genet* 2014;46:837–843.
26. Souza RF, Huo X, Mittal V, Schuler CM, Carmack SW, Zhang HY, Zhang X, Yu C, Hormi-Carver K, Genta RM, Spechler SJ. Gastroesophageal reflux might cause esophagitis through a cytokine-mediated mechanism rather than caustic acid injury. *Gastroenterology* 2009;137:1776–1784.
27. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med* 2015;21:677–687.
28. Henao-Mejia J, Elinav E, Strowig T, Flavell RA. Inflammasomes: far beyond inflammation. *Nat Immunol* 2012;13:321–324.
29. Kasza A. IL-1 and EGF regulate expression of genes important in inflammation and cancer. *Cytokine* 2013;62:22–33.
30. Zitvogel L, Kepp O, Galluzzi L, Kroemer G. Inflammasomes in carcinogenesis and anticancer immune responses. *Nat Immunol* 2012;13:343–351.
31. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- β . *Mol Cell* 2002;10:417–426.
32. He W, Yuan T, Maedler K. Macrophage-associated pro-inflammatory state in human islets from obese individuals. *Nutr Diabetes* 2019;9:36.
33. Nemetz A, Nosti-Escanilla MP, Molnar T, Kope A, Kovacs A, Feher J, Tulassay Z, Nagy F, Garcia-Gonzalez MA, Pena AS. IL1B gene polymorphisms influence the course and severity of inflammatory bowel disease. *Immunogenetics* 1999;49:527–531.
34. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, Betz KS, Penz-Oesterreicher M, Bjorkdahl O, Fox JG, Wang TC. Overexpression of interleukin-1 β induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 2008;14:408–419.
35. Jang JH, Kim DH, Lim JM, Lee JW, Jeong SJ, Kim KP, Surh YJ. Breast cancer cell-derived soluble CD44 promotes tumor progression by triggering macrophage IL1 β production. *Cancer Res* 2020;80:1342–1356.
36. Wong CC, Baum J, Silvestro A, Beste MT, Bharani-Dharan B, Xu S, Wang YA, Wang X, Prescott MF, Krajcovich L, Dugan M, Ridker PM, Martin AM, Svensson EC. Inhibition of IL1 β by canakinumab may be effective against diverse molecular subtypes of lung cancer: an exploratory analysis of the CANTOS trial. *Cancer Res* 2020;80:5597–5605.
37. Fitzgerald RC, Abdalla S, Onwuegbusi BA, Sirieix P, Saeed IT, Burnham WR, Farthing MJ. Inflammatory gradient in Barrett's oesophagus: implications for disease complications. *Gut* 2002;51:316–322.
38. Abdel-Latif MM, O'Riordan J, Windle HJ, Carton E, Ravi N, Kelleher D, Reynolds JV. NF- κ B activation in esophageal adenocarcinoma: relationship to Barrett's metaplasia, survival, and response to neoadjuvant chemoradiotherapy. *Ann Surg* 2004;239:491–500.
39. Duggan SP, Behan FM, Kirca M, Smith S, Reynolds JV, Long A, Kelleher D. An integrative genomic approach in oesophageal cells identifies TRB3 as a bile acid responsive gene, downregulated in Barrett's oesophagus, which regulates NF- κ B activation and cytokine levels. *Carcinogenesis* 2010;31:936–945.
40. Duggan SP, Garry C, Behan FM, Phipps S, Kudo H, Kirca M, Zaheer A, McGarrigle S, Reynolds JV, Goldin R, Kalloger SE, Schaeffer DF, Long A, Strid J, Kelleher D. siRNA library screening identifies a drug-gable immune-signature driving esophageal adenocarcinoma cell growth. *Cell Mol Gastroenterol Hepatol* 2018;5:569–590.
41. Quante M, Bhagat G, Abrams JA, Marache F, Good P, Lee MD, Lee Y, Friedman R, Asfaha S, Dubeykovskaya Z, Mahmood U, Figueiredo JL, Kitajewski J, Shawber C, Lightdale CJ, Rustgi AK, Wang TC. Bile acid and inflammation activate gastric cardia stem cells in a mouse model of Barrett-like metaplasia. *Cancer Cell* 2012;21:36–51.
42. Kapoor H, Lohani KR, Lee TH, Agrawal DK, Mittal SK. Animal models of Barrett's esophagus and esophageal adenocarcinoma-past, present, and future. *Clin Transl Sci* 2015;8:841–847.
43. Spechler SJ. Of mice and men and metaplasia. *Cell Mol Gastroenterol Hepatol* 2017;4:183–184.
44. Nowicki-Osuch K, Zhuang L, Jammula S, Bleaney CW, Mahbubani KT, Devonshire G, Katz-Summercorn A, Eling N, Wilbrey-Clark A, Madisson E, Gamble J, Di Pietro M, O'Donovan M, Meyer KB, Saeb-Parsy K, Sharrocks AD, Teichmann SA, Marioni JC, Fitzgerald RC. Molecular phenotyping reveals the identity of Barrett's esophagus and its malignant transition. *Science* 2021;373:760–767.

45. Gillen P, Keeling P, Byrne PJ, West AB, Hennessy TP. Experimental columnar metaplasia in the canine oesophagus. *Br J Surg* 1988;75:113–115.
46. Tselepis C, Perry I, Dawson C, Hardy R, Darnton SJ, McConkey C, Stuart RC, Wright N, Harrison R, Jankowski JA. Tumour necrosis factor- α in Barrett's oesophagus: a potential novel mechanism of action. *Oncogene* 2002;21:6071–6081.
47. Duggan SP, Gallagher WM, Fox EJ, Abdel-Latif MM, Reynolds JV, Kelleher D. Low pH induces co-ordinate regulation of gene expression in oesophageal cells. *Carcinogenesis* 2006;27:319–327.
48. Jimenez P, Piazuelo E, Cebrian C, Ortego J, Strunk M, Garcia-Gonzalez MA, Santander S, Alcedo J, Lanás A. Prostaglandin EP2 receptor expression is increased in Barrett's oesophagus and oesophageal adenocarcinoma. *Aliment Pharmacol Ther* 2010;31:440–451.
49. Timme S, Ihde S, Fichter CD, Waehle V, Bogatyreva L, Atanasov K, Kohler I, Schopflin A, Geddert H, Faller G, Klimstra D, Tang L, Reinheckel T, Hauschke D, Busch H, Boerries M, Werner M, Lassmann S. STAT3 expression, activity and functional consequences of STAT3 inhibition in esophageal squamous cell carcinomas and Barrett's adenocarcinomas. *Oncogene* 2014;33:3256–3266.
50. Lagisetty KH, McEwen DP, Nancarrow DJ, Schiebel JG, Ferrer-Torres D, Ray D, Frankel TL, Lin J, Chang AC, Kresty LA, Beer DG. Immune determinants of Barrett's progression to esophageal adenocarcinoma. *JCI Insight* 2021;6:e143888.
51. Souza RF. Reflux esophagitis and its role in the pathogenesis of Barrett's metaplasia. *J Gastroenterol* 2017;52:767–776.
52. Duggan SP, Behan FM, Kirca M, Zaheer A, McGarrigle SA, Reynolds JV, Vaz GM, Senge MO, Kelleher D. The characterization of an intestine-like genomic signature maintained during Barrett's-associated adenocarcinogenesis reveals an NR5A2-mediated promotion of cancer cell survival. *Sci Rep* 2016;6:32638.
53. Kavanagh ME, O'Sullivan KE, O'Hanlon C, O'Sullivan JN, Lysaght J, Reynolds JV. The esophagitis to adenocarcinoma sequence; the role of inflammation. *Cancer Lett* 2014;345:182–189.
54. Morris CD, Armstrong GR, Bigley G, Green H, Attwood SE. Cyclooxygenase-2 expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. *Am J Gastroenterol* 2001;96:990–996.
55. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A* 1975;72:3666–3670.
56. Gough P, Myles IA. Tumor necrosis factor receptors: pleiotropic signaling complexes and their differential effects. *Front Immunol* 2020;11:585880.
57. Kolb WP, Granger GA. Lymphocyte in vitro cytotoxicity: characterization of human lymphotoxin. *Proc Natl Acad Sci U S A* 1968;61:1250–1255.
58. McCall JL, Yun K, Funamoto S, Parry BR. In vivo immunohistochemical identification of tumor necrosis factor/cachectin in human lymphoid tissue. *Am J Pathol* 1989;135:421–425.
59. Leclerc M, Naserian S, Pilon C, Thiolat A, Martin GH, Pouchy C, Dominique C, Belkacemi Y, Charlotte F, Maury S, Salomon BL, Cohen JL. Control of GVHD by regulatory T cells depends on TNF produced by T cells and TNFR2 expressed by regulatory T cells. *Blood* 2016;128:1651–1659.
60. Olszewski MB, Groot AJ, Dastyh J, Knol EF. TNF trafficking to human mast cell granules: mature chain-dependent endocytosis. *J Immunol* 2007;178:5701–5709.
61. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984;312:724–729.
62. Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: live or let die. *Nat Rev Immunol* 2015;15:362–374.
63. Vince JE, Pantaki D, Feltham R, Mace PD, Cordier SM, Schmukle AC, Davidson AJ, Callus BA, Wong WW, Gentle IE, Carter H, Lee EF, Walczak H, Day CL, Vaux DL, Silke J. TRAF2 must bind to cellular inhibitors of apoptosis for tumor necrosis factor (tnf) to efficiently activate nf- κ b and to prevent tnf-induced apoptosis. *J Biol Chem* 2009;284:35906–35915.
64. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 1996;84:299–308.
65. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF- κ B activation. *Cell* 1995;81:495–504.
66. Sedger LM, McDermott MF. TNF and TNF-receptors: from mediators of cell death and inflammation to therapeutic giants - past, present and future. *Cytokine Growth Factor Rev* 2014;25:453–472.
67. Shu HB, Takeuchi M, Goeddel DV. The tumor necrosis factor receptor 2 signal transducers TRAF2 and c-IAP1 are components of the tumor necrosis factor receptor 1 signaling complex. *Proc Natl Acad Sci U S A* 1996;93:13973–13978.
68. Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, Cruciat C, Eberhard D, Gagneur J, Ghidelli S, Hopf C, Huhse B, Mangano R, Michon AM, Schirle M, Schlegl J, Schwab M, Stein MA, Bauer A, Casari G, Drewes G, Gavin AC, Jackson DB, Joberty G, Neubauer G, Rick J, Kuster B, Superti-Furga G. A physical and functional map of the human TNF- α /NF- κ B signal transduction pathway. *Nat Cell Biol* 2004;6:97–105.
69. Coskun M, Olsen AK, Bzorek M, Holck S, Engel UH, Nielsen OH, Troelsen JT. Involvement of CDX2 in the cross talk between TNF- α and Wnt signaling pathway in the colon cancer cell line Caco-2. *Carcinogenesis* 2014;35:1185–1192.
70. Hadisaputri YE, Miyazaki T, Suzuki S, Yokobori T, Kobayashi T, Tanaka N, Inose T, Sohda M, Kuwano H. TNFAIP8 overexpression: clinical relevance to esophageal squamous cell carcinoma. *Ann Surg Oncol* 2012;19(Suppl 3):S589–S596.

71. Song L, Gong H, Lin C, Wang C, Liu L, Wu J, Li M, Li J. Flotillin-1 promotes tumor necrosis factor- α receptor signaling and activation of NF- κ B in esophageal squamous cell carcinoma cells. *Gastroenterology* 2012; 143:995–1005 e12.
72. Cook MB, Barnett MJ, Bock CH, Cross AJ, Goodman PJ, Goodman GE, Haiman CA, Khaw KT, McCullough ML, Newton CC, Boutron-Ruault MC, Lund E, Rutegard M, Thornquist MD, Spriggs M, Giffen C, Freedman ND, Kemp T, Kroenke CH, Le Marchand L, Park JY, Simon M, Wilkens LR, Pinto L, Hildesheim A, Campbell PT. Prediagnostic circulating markers of inflammation and risk of oesophageal adenocarcinoma: a study within the National Cancer Institute Cohort Consortium. *Gut* 2019;68:960–968.
73. Peng DF, Hu TL, Soutto M, Belkhiri A, El-Rifai W. Loss of glutathione peroxidase 7 promotes TNF- α -induced NF- κ B activation in Barrett's carcinogenesis. *Carcinogenesis* 2014;35:1620–1628.
74. Looby E, Abdel-Latif MM, Athie-Morales V, Duggan S, Long A, Kelleher D. Deoxycholate induces COX-2 expression via Erk1/2-, p38-MAPK and AP-1-dependent mechanisms in esophageal cancer cells. *BMC Cancer* 2009;9:190.
75. Chemnitzer O, Gotzel K, Maurer L, Dietrich A, Eichfeld U, Lyros O, Jansen-Winkel B, Hoffmeister A, Gockel I, Thieme R. Response to TNF- α is increasing along with the progression in Barrett's esophagus. *Dig Dis Sci* 2017;62:3391–3401.
76. Morrissey ME, Byrne R, Nulty C, McCabe NH, Lynam-Lennon N, Butler CT, Kennedy S, O'Toole D, Larkin J, McCormick P, Mehigan B, Cathcart MC, Lysaght J, Reynolds JV, Ryan EJ, Dunne MR, O'Sullivan J. The tumour microenvironment of the upper and lower gastrointestinal tract differentially influences dendritic cell maturation. *BMC Cancer* 2020;20:566.
77. Zhan Y, Xu C, Liu Z, Yang Y, Tan S, Yang Y, Jiang J, Liu H, Chen J, Wu B. beta-Arrestin1 inhibits chemotherapy-induced intestinal stem cell apoptosis and mucositis. *Cell Death Dis* 2016;7:e2229.
78. Zhan T, Ambrosi G, Wandmacher AM, Rauscher B, Betge J, Rindtorff N, Haussler RS, Hinsenkamp I, Bamberg L, Hessling B, Muller-Decker K, Erdmann G, Burgermeister E, Ebert MP, Boutros M. MEK inhibitors activate Wnt signalling and induce stem cell plasticity in colorectal cancer. *Nat Commun* 2019;10:2197.
79. He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. Identification of c-MYC as a target of the APC pathway. *Science* 1998;281:1509–1512.
80. Moon H, White AC, Borowsky AD. New insights into the functions of Cox-2 in skin and esophageal malignancies. *Exp Mol Med* 2020;52:538–547.
81. Park MH, Hong JT. Roles of NF- κ B in cancer and inflammatory diseases and their therapeutic approaches. *Cells* 2016;5:15.
82. Arenzana-Seisdedos F, Thompson J, Rodriguez MS, Bachelier F, Thomas D, Hay RT. Inducible nuclear expression of newly synthesized I kappa B alpha negatively regulates DNA-binding and transcriptional activities of NF- κ B. *Mol Cell Biol* 1995; 15:2689–2696.
83. Arenzana-Seisdedos F, Turpin P, Rodriguez M, Thomas D, Hay RT, Virelizier JL, Dargemont C. Nuclear localization of I kappa B alpha promotes active transport of NF- κ B from the nucleus to the cytoplasm. *J Cell Sci* 1997;110:369–378.
84. Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 1986; 46:705–716.
85. Sun SC. Non-canonical NF- κ B signaling pathway. *Cell Res* 2011;21:71–85.
86. Xiao G, Harhaj EW, Sun SC. NF- κ B-inducing kinase regulates the processing of NF- κ B2 p100. *Mol Cell* 2001;7:401–409.
87. Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. RANK is essential for osteoclast and lymph node development. *Genes Dev* 1999;13:2412–2424.
88. Koike R, Nishimura T, Yasumizu R, Tanaka H, Hataba Y, Hataba Y, Watanabe T, Miyawaki S, Miyasaka M. The splenic marginal zone is absent in alymphoplastic aly mutant mice. *Eur J Immunol* 1996;26:669–675.
89. Yilmaz ZB, Weih DS, Sivakumar V, Weih F. RelB is required for Peyer's patch development: differential regulation of p52-RelB by lymphotoxin and TNF. *EMBO J* 2003;22:121–130.
90. Bird TA, Schooley K, Dower SK, Hagen H, Virca GD. Activation of nuclear transcription factor NF- κ B by interleukin-1 is accompanied by casein kinase II-mediated phosphorylation of the p65 subunit. *J Biol Chem* 1997;272:32606–32612.
91. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF- κ B: variations on a common theme. *Cell Mol Life Sci* 2008;65:2964–2978.
92. Wang D, Baldwin AS Jr. Activation of nuclear factor- κ B-dependent transcription by tumor necrosis factor- α is mediated through phosphorylation of RelA/p65 on serine 529. *J Biol Chem* 1998; 273:29411–29416.
93. Wang D, Westerheide SD, Hanson JL, Baldwin AS Jr. Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. *J Biol Chem* 2000;275:32592–32597.
94. Hiscott J, Marois J, Garoufalidis J, D'Addario M, Roulston A, Kwan I, Pepin N, Lacoste J, Nguyen H, Bensi G. Characterization of a functional NF- κ B site in the human interleukin 1 beta promoter: evidence for a positive autoregulatory loop. *Mol Cell Biol* 1993; 13:6231–6240.
95. Kunsch C, Rosen CA. NF- κ B subunit-specific regulation of the interleukin-8 promoter. *Mol Cell Biol* 1993;13:6137–6146.
96. Collart MA, Baeuerle P, Vassalli P. Regulation of tumor necrosis factor alpha transcription in macrophages: involvement of four kappa B-like motifs and of

- constitutive and inducible forms of NF-kappa B. *Mol Cell Biol* 1990;10:1498–1506.
97. Shakhov AN, Kuprash DV, Azizov MM, Jongeneel CV, Nedospasov SA. Structural analysis of the rabbit TNF locus, containing the genes encoding TNF-beta (lymphotoxin) and TNF-alpha (tumor necrosis factor). *Gene* 1990;95:215–221.
 98. Frankell AM, Jammula S, Li X, Contino G, Killcoyne S, Abbas S, Perner J, Bower L, Devonshire G, Ococks E, Grehan N, Mok J, O'Donovan M, MacRae S, Eldridge MD, Tavare S, Oesophageal Cancer C, Molecular Stratification C, Fitzgerald RC. The landscape of selection in 551 esophageal adenocarcinomas defines genomic biomarkers for the clinic. *Nat Genet* 2019;51:506–516.
 99. Bruzzese F, Hagglof C, Leone A, Sjoberg E, Roca MS, Kiflemariam S, Sjoblom T, Hammarsten P, Egevad L, Bergh A, Ostman A, Budillon A, Augsten M. Local and systemic protumorigenic effects of cancer-associated fibroblast-derived GDF15. *Cancer Res* 2014;74:3408–3417.
 100. Ishige T, Nishimura M, Satoh M, Fujimoto M, Fukuyo M, Semba T, Kado S, Tsuchida S, Sawai S, Matsushita K, Togawa A, Matsubara H, Kaneda A, Nomura F. Combined secretomics and transcriptomics revealed cancer-derived GDF15 is involved in diffuse-type gastric cancer progression and fibroblast activation. *Sci Rep* 2016;6:21681.
 101. McKarns SC, Schwartz RH. Distinct effects of TGF-beta 1 on CD4+ and CD8+ T cell survival, division, and IL-2 production: a role for T cell intrinsic Smad3. *J Immunol* 2005;174:2071–2083.
 102. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor RORgamma directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006;126:1121–1133.
 103. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G, Vollmar P, Stritesky GL, Kaplan MH, Waisman A, Kuchroo VK, Oukka M. IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. *Proc Natl Acad Sci U S A* 2008;105:18460–18465.
 104. Thomas DA, Massague J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* 2005;8:369–380.
 105. Diskin B, Adam S, Cassini MF, Sanchez G, Liria M, Aykut B, Buttar C, Li E, Sundberg B, Salas RD, Chen R, Wang J, Kim M, Farooq MS, Nguy S, Fedele C, Tang KH, Chen T, Wang W, Hundeyin M, Rossi JAK, Kurz E, Haq MIU, Karlen J, Kruger E, Sekendiz Z, Wu D, Shadaloey SAA, Baptiste G, Werba G, Selvaraj S, Loomis C, Wong KK, Leinwand J, Miller G. PD-L1 engagement on T cells promotes self-tolerance and suppression of neighboring macrophages and effector T cells in cancer. *Nat Immunol* 2020;21:442–454.
 106. David CJ, Huang YH, Chen M, Su J, Zou Y, Bardeesy N, Iacobuzio-Donahue CA, Massague J. TGF-beta tumor suppression through a lethal EMT. *Cell* 2016;164:1015–1030.
 107. Cai Z, Zhang W, Yang F, Yu L, Yu Z, Pan J, Wang L, Cao X, Wang J. Immunosuppressive exosomes from TGF-beta1 gene-modified dendritic cells attenuate Th17-mediated inflammatory autoimmune disease by inducing regulatory T cells. *Cell Res* 2012;22:607–610.
 108. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF, Littman DR. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgamma function. *Nature* 2008;453:236–240.
 109. Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, Rudensky AY. CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science* 2009;326:986–991.
 110. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Ma L, Watowich SS, Jetten AM, Tian Q, Dong C. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity* 2009;30:576–587.
 111. Gotovac JR, Kader T, Milne JV, Fujihara KM, Lara-Gonzalez LE, Gorringer KL, Kalimuthu SN, Jayawardana MW, Duong CP, Phillips WA, Clemons NJ. Loss of SMAD4 is sufficient to promote tumorigenesis in a model of dysplastic Barrett's esophagus. *Cell Mol Gastroenterol Hepatol* 2021;12:689–713.
 112. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* 2006;441:231–234.
 113. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007;317:256–260.
 114. Onwuegbusi BA, Aitchison A, Chin SF, Kranjac T, Mills I, Huang Y, Lao-Sirieix P, Caldas C, Fitzgerald RC. Impaired transforming growth factor beta signalling in Barrett's carcinogenesis due to frequent SMAD4 inactivation. *Gut* 2006;55:764–774.
 115. Singhi AD, Foxwell TJ, Nason K, Cressman KL, McGrath KM, Sun W, Bahary N, Zeh HJ, Levy RM, Luketich JD, Davison JM. Smad4 loss in esophageal adenocarcinoma is associated with an increased propensity for disease recurrence and poor survival. *Am J Surg Pathol* 2015;39:487–495.
 116. Dvorak K, Chavarria M, Payne CM, Ramsey L, Crowley-Weber C, Dvorakova B, Dvorak B, Bernstein H, Holubec H, Sampliner RE, Bernstein C, Prasad A, Green SB, Garewal H. Activation of the interleukin-6/STAT3 antiapoptotic pathway in esophageal cells by bile acids and low pH: relevance to Barrett's esophagus. *Clin Cancer Res* 2007;13:5305–5313.
 117. Carpenter RL, Lo HW. STAT3 target genes relevant to human cancers. *Cancers (Basel)* 2014;6:897–925.
 118. Nishihara M, Ogura H, Ueda N, Tsuruoka M, Kitabayashi C, Tsuji F, Aono H, Ishihara K, Huseby E, Betz UA, Murakami M, Hirano T. IL-6-gp130-STAT3 in T cells directs the development of IL-17+ Th with a minimum effect on that of Treg in the steady state. *Int Immunol* 2007;19:695–702.

119. Jiang C, Zhang Q, Shanti RM, Shi S, Chang TH, Carrasco L, Alawi F, Le AD. Mesenchymal stromal cell-derived interleukin-6 promotes epithelial-mesenchymal transition and acquisition of epithelial stem-like cell properties in ameloblastoma epithelial cells. *Stem Cells* 2017;35:2083–2094.
120. Zhang HY, Zhang Q, Zhang X, Yu C, Huo X, Cheng E, Wang DH, Spechler SJ, Souza RF. Cancer-related inflammation and Barrett's carcinogenesis: interleukin-6 and STAT3 mediate apoptotic resistance in transformed Barrett's cells. *Am J Physiol Gastrointest Liver Physiol* 2011;300:G454–G460.
121. Zhang N, Bevan MJ. TGF-beta signaling to T cells inhibits autoimmunity during lymphopenia-driven proliferation. *Nat Immunol* 2012;13:667–673.
122. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. *Semin Immunopathol* 2013;35:123–137.
123. Wang D, DuBois RN. Role of prostanoids in gastrointestinal cancer. *J Clin Invest* 2018;128:2732–2742.
124. Kaur BS, Triadafilopoulos G. Acid- and bile-induced PGE(2) release and hyperproliferation in Barrett's esophagus are COX-2 and PKC-epsilon dependent. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G327–G334.
125. Majewski M, Wallner G, Sarosiek J. The potential pathogenetic role of esophageal prostaglandins in patients with Barrett's esophagus: its clinical implication: presidential poster: 87. *Am Coll Gastroenterol* 2013:108.
126. Zhang M, Liu Q, Mi S, Liang X, Zhang Z, Su X, Liu J, Chen Y, Wang M, Zhang Y, Guo F, Zhang Z, Yang R. Both miR-17-5p and miR-20a alleviate suppressive potential of myeloid-derived suppressor cells by modulating STAT3 expression. *J Immunol* 2011;186:4716–4724.
127. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 2015;16:448–457.
128. Jones SA, Jenkins BJ. Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. *Nat Rev Immunol* 2018;18:773–789.
129. Kang S, Tanaka T, Narazaki M, Kishimoto T. Targeting interleukin-6 signaling in clinic. *Immunity* 2019;50:1007–1023.
130. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014;6:a016295.
131. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol* 2018;15:234–248.
132. Auernhammer CJ, Melmed S. Leukemia-inhibitory factor—neuroimmune modulator of endocrine function*. *Endocr Rev* 2000;21:313–345.
133. Bauer S, Patterson PH. Leukemia inhibitory factor promotes neural stem cell self-renewal in the adult brain. *J Neurosci* 2006;26:12089–12099.
134. Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. *Proc Natl Acad Sci U S A* 1996;93:3115–3120.
135. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Mol Cell* 2010;39:493–506.
136. Bhat AA, Lu H, Soutto M, Capobianco A, Rai P, Zaika A, El-Rifai W. Exposure of Barrett's and esophageal adenocarcinoma cells to bile acids activates EGFR-STAT3 signaling axis via induction of APE1. *Oncogene* 2018;37:6011–6024.
137. 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* 2016;65:1416–1426.
138. Chen L, Huang M, Plummer J, Pan J, Jiang YY, Yang Q, Silva TC, Gull N, Chen S, Ding LW, An O, Yang H, Cheng Y, Said JW, Doan N, Dinjens WN, Waters KM, Tuli R, Gayther SA, Klempner SJ, Berman BP, Meltzer SJ, Lin DC, Koeffler HP. Master transcription factors form interconnected circuitry and orchestrate transcriptional networks in oesophageal adenocarcinoma. *Gut* 2020;69:630–640.
139. Kaz AM, Grady WM, Stachler MD, Bass AJ. Genetic and epigenetic alterations in Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterol Clin North Am* 2015;44:473–489.
140. Lin L, Bass AJ, Lockwood WW, Wang Z, Silvers AL, Thomas DG, Chang AC, Lin J, Orringer MB, Li W, Glover TW, Giordano TJ, Lam WL, Meyerson M, Beer DG. Activation of GATA binding protein 6 (GATA6) sustains oncogenic lineage-survival in esophageal adenocarcinoma. *Proc Natl Acad Sci U S A* 2012;109:4251–4256.
141. Phipps SM, Garry CE, Kamal S, Johnson JD, Gilmer J, Long A, Kelleher D, Duggan SP. High Content imaging of Barrett's-associated high-grade dysplasia cells after siRNA library screening reveals acid-responsive regulators of cellular transitions. *Cell Mol Gastroenterol Hepatol* 2020;10:601–622.
142. van Baal JW, Verbeek RE, Bus P, Fassan M, Souza RF, Rugge M, ten Kate FJ, Vleggaar FP, Siersema PD. microRNA-145 in Barrett's oesophagus: regulating BMP4 signalling via GATA6. *Gut* 2013;62:664–675.
143. Cao W, Peters JH, Nieman D, Sharma M, Watson T, Yu J. Macrophage subtype predicts lymph node metastasis in oesophageal adenocarcinoma and promotes cancer cell invasion in vitro. *Br J Cancer* 2015;113:738–746.
144. Duluc D, Delneste Y, Tan F, Moles MP, Grimaud L, Lenoir J, Preisser L, Anegon I, Catala L, Ibrah N, Descamps P, Gamelin E, Gascan H, Hebbar M, Jeannin P. Tumor-associated leukemia inhibitory factor and IL-6 skew monocyte differentiation into tumor-associated macrophage-like cells. *Blood* 2007;110:4319–4330.

145. Massague J. TGFbeta in Cancer. *Cell* 2008; 134:215–230.
146. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003; 113:685–700.
147. Li MO, Flavell RA. TGF-beta: a master of all T cell trades. *Cell* 2008;134:392–404.
148. Zhang YE. Non-Smad pathways in TGF-beta signaling. *Cell Res* 2009;19:128–139.
149. Kalli M, Minia A, Pliaka V, Fotis C, Alexopoulos LG, Stylianopoulos T. Solid stress-induced migration is mediated by GDF15 through Akt pathway activation in pancreatic cancer cells. *Sci Rep* 2019;9:978.
150. Rochette L, Zeller M, Cottin Y, Vergely C. Insights into mechanisms of GDF15 and receptor GFRAL: therapeutic targets. *Trends Endocrinol Metab* 2020;31:939–951.
151. Suriben R, Chen M, Higbee J, Oeffinger J, Ventura R, Li B, Mondal K, Gao Z, Ayupova D, Taskar P, Li D, Starck SR, Chen HH, McEntee M, Katewa SD, Phung V, Wang M, Kekatpure A, Lakshminarasimhan D, White A, Olland A, Haldankar R, Solloway MJ, Hsu JY, Wang Y, Tang J, Lindhout DA, Allan BB. Antibody-mediated inhibition of GDF15-GFRAL activity reverses cancer cachexia in mice. *Nat Med* 2020;26:1264–1270.
152. Yang L, Chang CC, Sun Z, Madsen D, Zhu H, Padkjaer SB, Wu X, Huang T, Hultman K, Paulsen SJ, Wang J, Bugge A, Frantzen JB, Norgaard P, Jeppesen JF, Yang Z, Secher A, Chen H, Li X, John LM, Shan B, He Z, Gao X, Su J, Hansen KT, Yang W, Jorgensen SB. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. *Nat Med* 2017;23:1158–1166.
153. Prud'homme GJ. Pathobiology of transforming growth factor beta in cancer, fibrosis and immunologic disease, and therapeutic considerations. *Lab Invest* 2007; 87:1077–1091.
154. McKarns SC, Schwartz RH, Kaminski NE. Smad3 is essential for TGF-beta 1 to suppress IL-2 production and TCR-induced proliferation, but not IL-2-induced proliferation. *J Immunol* 2004;172:4275–4284.
155. Jun E, Song AY, Choi JW, Lee HH, Kim MY, Ko DH, Kang HJ, Kim SW, Bryceson Y, Kim SC, Kim HS. Progressive impairment of NK cell cytotoxic degranulation is associated with TGF-beta1 deregulation and disease progression in pancreatic cancer. *Front Immunol* 2019; 10:1354.
156. Aparicio-Pages MN, Verspaget HW, Pena AS, Lamers CB. Natural killer cell activity in patients with adenocarcinoma in the upper gastrointestinal tract. *J Clin Lab Immunol* 1991;35:27–32.
157. Daster S, Eppenberger-Castori S, Mele V, Schafer HM, Schmid L, Weixler B, Soysal SD, Drosner RA, Spagnoli GC, Kettelhack C, Oertli D, Terracciano L, Tornillo L, von Holzen U. Low expression of programmed death 1 (PD-1), PD-1 ligand 1 (PD-L1), and low CD8+ T lymphocyte infiltration identify a subgroup of patients with gastric and esophageal adenocarcinoma with severe prognosis. *Front Med (Lausanne)* 2020;7:144.
158. Schumacher K, Haensch W, Röefzaad C, Schlag PM. Prognostic significance of activated CD8+ T cell infiltrations within esophageal carcinomas. *Cancer Res* 2001;61:3932–3936.
159. Hao D, He S, Harada K, Pizzi MP, Lu Y, Guan P, Chen L, Wang R, Zhang S, Sewastjanow-Silva M, Abdelhakeem A, Shanbhag N, Bhutani M, Han G, Lee JH, Zhao S, Weston B, Blum Murphy M, Waters R, Estrella JS, Roy-Chowdhuri S, Gan Q, Lee JS, Peng G, Hanash SM, Calin GA, Song X, Zhang J, Song S, Wang L, Ajani JA. Integrated genomic profiling and modelling for risk stratification in patients with advanced oesophagogastric adenocarcinoma. *Gut* 2021; 70:2055–2065.
160. Lee CR, Kwak Y, Yang T, Han JH, Park SH, Ye MB, Lee W, Sim KY, Kang JA, Kim YC, Mazmanian SK, Park SG. Myeloid-derived suppressor cells are controlled by regulatory T cells via TGF-beta during murine colitis. *Cell Rep* 2016;17:3219–3232.
161. Blum AE, Venkitachalam S, Ravillah D, Chelluboyina AK, Kieber-Emmons AM, Ravi L, Kresak A, Chandar AK, Markowitz SD, Canto MI, Wang JS, Shaheen NJ, Guo Y, Shyr Y, Willis JE, Chak A, Varadan V, Guda K. Systems biology analyses show hyperactivation of transforming growth factor-beta and JNK signaling pathways in esophageal cancer. *Gastroenterology* 2019;156:1761–1774.
162. Means AL, Freeman TJ, Zhu J, Woodbury LG, Marincola-Smith P, Wu C, Meyer AR, Weaver CJ, Padmanabhan C, An H, Zi J, Wessinger BC, Chaturvedi R, Brown TD, Deane NG, Coffey RJ, Wilson KT, Smith JJ, Sawyers CL, Goldenring JR, Novitskiy SV, Washington MK, Shi C, Beauchamp RD. Epithelial Smad4 deletion up-regulates inflammation and promotes inflammation-associated cancer. *Cell Mol Gastroenterol Hepatol* 2018;6:257–276.
163. Battle E, Massague J. Transforming growth factor-beta signaling in immunity and cancer. *Immunity* 2019; 50:924–940.
164. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006;441:235–238.
165. Bobak DA, Gaither TA, Frank MM, Tenner AJ. Modulation of FcR function by complement: subcomponent C1q enhances the phagocytosis of IgG-opsonized targets by human monocytes and culture-derived macrophages. *J Immunol* 1987;138:1150–1156.
166. Nepomuceno RR, Tenner AJ. C1qRP, the C1q receptor that enhances phagocytosis, is detected specifically in human cells of myeloid lineage, endothelial cells, and platelets. *J Immunol* 1998;160:1929–1935.
167. Schwaeble W, Schafer MK, Petry F, Fink T, Knebel D, Weihe E, Loos M. Follicular dendritic cells, interdigitating cells, and cells of the monocyte-macrophage lineage are the C1q-producing sources in the spleen. Identification of specific cell types by in situ hybridization and immunohistochemical analysis. *J Immunol* 1995; 155:4971–4978.
168. Lu JH, Teh BK, Wang L, Wang YN, Tan YS, Lai MC, Reid KB. The classical and regulatory functions of C1q in immunity and autoimmunity. *Cell Mol Immunol* 2008; 5:9–21.

169. Yonemasu K, Kitajima H, Tanabe S, Ochi T, Shinkai H. Effect of age on C1q and C3 levels in human serum and their presence in colostrum. *Immunology* 1978; 35:523–530.
170. Rossen RD, Reisberg MA, Hersh EM, Gutterman JU. The C1q binding test for soluble immune complexes: clinical correlations obtained in patients with cancer. *J Natl Cancer Inst* 1977;58:1205–1215.
171. Niwa H, Ogawa K, Shimosato D, Adachi K. A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature* 2009;460:118–122.
172. Chong IY, Aronson L, Bryant H, Gulati A, Campbell J, Elliott R, Pettitt S, Wilkerson P, Lambros MB, Reis-Filho JS, Ramessur A, Davidson M, Chau I, Cunningham D, Ashworth A, Lord CJ. Mapping genetic vulnerabilities reveals BTK as a novel therapeutic target in oesophageal cancer. *Gut* 2018;67:1780–1792.
173. Naito AT, Sumida T, Nomura S, Liu ML, Higo T, Nakagawa A, Okada K, Sakai T, Hashimoto A, Hara Y, Shimizu I, Zhu W, Toko H, Katada A, Akazawa H, Oka T, Lee JK, Minamino T, Nagai T, Walsh K, Kikuchi A, Matsumoto M, Botto M, Shiojima I, Komuro I. Complement C1q activates canonical Wnt signaling and promotes aging-related phenotypes. *Cell* 2012; 149:1298–1313.
174. Jung YS, Park JI. Wnt signaling in cancer: therapeutic targeting of Wnt signaling beyond beta-catenin and the destruction complex. *Exp Mol Med* 2020;52:183–191.
175. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell* 2009;17:9–26.
176. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136: 2348–2357.
177. Rengarajan J, Szabo SJ, Glimcher LH. Transcriptional regulation of Th1/Th2 polarization. *Immunol Today* 2000; 21:479–483.
178. 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;50:451–459.
179. Shan J, Oshima T, Farre R, Fukui H, Watari J, Miwa H. IL-4 induces columnar-like differentiation of esophageal squamous epithelium through JAK/PI3K pathway: possible role in pathogenesis of Barrett's esophagus. *Am J Physiol Gastrointest Liver Physiol* 2014; 306:G641–G649.
180. Kavanagh ME, Conroy MJ, Clarke NE, Gilmartin NT, O'Sullivan KE, Feighery R, MacCarthy F, O'Toole D, Ravi N, Reynolds JV, O'Sullivan J, Lysaght J. Impact of the inflammatory microenvironment on T-cell phenotype in the progression from reflux oesophagitis to Barrett oesophagus and oesophageal adenocarcinoma. *Cancer Lett* 2016;370:117–124.
181. Lind A, Siersema PD, Kusters JG, Van der Linden JA, Knol EF, Koenderman L. The immune cell composition in Barrett's metaplastic tissue resembles that in normal duodenal tissue. *PLoS One* 2012;7:e33899.
182. Lipka S, Kumar A, Richter JE. PPI trial for eosinophilic esophagitis: chaos in the community. *J Clin Gastroenterol* 2018;52:590–595.
183. Dellon ES, Liacouras CA, Molina-Infante J, Furuta GT, Speigel JM, Zevit N, Spechler SJ, Attwood SE, Straumann A, Aceves SS, Alexander JA, Atkins D, Arva NC, Blanchard C, Bonis PA, Book WM, Capocelli KE, Chehade M, Cheng E, Collins MH, Davis CM, Dias JA, Di Lorenzo C, Dohil R, Dupont C, Falk GW, Ferreira CT, Fox A, Gonsalves NP, Gupta SK, Katzka DA, Kinoshita Y, Menard-Katcher C, Kodroff E, Metz DC, Miehke S, Muir AB, Mukkada VA, Murch S, Nurko S, Ohtsuka Y, Orel R, Papadopoulou A, Peterson KA, Philpott H, Putnam PE, Richter JE, Rosen R, Rothenberg ME, Schoepfer A, Scott MM, Shah N, Sheikh J, Souza RF, Strobel MJ, Talley NJ, Vaezi MF, Vandenplas Y, Vieira MC, Walker MM, Wechsler JB, Wershil BK, Wen T, Yang GY, Hirano I, Bredenoord AJ. Updated international consensus diagnostic criteria for eosinophilic esophagitis: proceedings of the AGREE conference. *Gastroenterology* 2018; 155:1022–1033 e10.
184. Lucendo AJ, Arias A, Molina-Infante J. Efficacy of proton pump inhibitor drugs for inducing clinical and histologic remission in patients with symptomatic esophageal eosinophilia: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2016;14:13–22 e1.
185. Dunbar KB, Agoston AT, Odze RD, Huo X, Pham TH, Cipher DJ, Castell DO, Genta RM, Souza RF, Spechler SJ. Association of acute gastroesophageal reflux disease with esophageal histologic changes. *JAMA* 2016;315:2104–2112.
186. Chambers JK, Saito T, Fukushima K, Kakuta S, Nakayama J, Ohno K, Tsujimoto H, Nakayama H, Uchida K. Adenocarcinoma of Barrett's esophagus in a dog. *J Toxicol Pathol* 2017;30:239–243.
187. Naranjo JD, Saldin LT, Sobieski E, Quijano LM, Hill RC, Chan PG, Torres C, Dziki JL, Cramer MC, Lee YC, Das R, Bajwa AK, Nossair R, Klimak M, Marchal L, Patel S, Velankar SS, Hansen KC, McGrath K, Badylak SF. Esophageal extracellular matrix hydrogel mitigates metaplastic change in a dog model of Barrett's esophagus. *Sci Adv* 2020;6:eaba4526.
188. Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, Timofeeva OA, Nealon C, Dakic A, Simic V, Haddad BR, Rhim JS, Dritschilo A, Riegel A, McBride A, Schlegel R. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am J Pathol* 2012;180:599–607.
189. Yamamoto Y, Wang X, Bertrand D, Kern F, Zhang T, Duleba M, Srivastava S, Khor CC, Hu Y, Wilson LH, Blaszyk H, Rolshud D, Teh M, Liu J, Howitt BE, Vincent M, Crum CP, Nagarajan N, Ho KY, McKeon F, Xian W. Mutational spectrum of Barrett's stem cells suggests paths to initiation of a precancerous lesion. *Nat Commun* 2016;7:10380.
190. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma,

- adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011;141:1762–1772.
191. Liu X, Cheng Y, Abraham JM, Wang Z, Wang Z, Ke X, Yan R, Shin EJ, Ngamruengphong S, Khashab MA, Zhang G, McNamara G, Ewald AJ, Lin D, Liu Z, Meltzer SJ. Modeling Wnt signaling by CRISPR-Cas9 genome editing recapitulates neoplasia in human Barrett epithelial organoids. *Cancer Lett* 2018;436:109–118.
192. Salahudeen AA, Li XN, Cantrell M, Kuo CJ. Gastrointestinal organoid cultures for functional evaluation of oncogenic loci. *J Clin Oncol* 2015;33:85.
193. Lo YH, Karlsson K, Kuo CJ. Applications of organoids for cancer biology and precision medicine. *Nat Cancer* 2020;1:761–773.
194. Davis Z, Felices M, Lenvik T, Badal S, Walker JT, Hinderlie P, Riley JL, Vallera DA, Blazar BR, Miller JS. Low-density PD-1 expression on resting human natural

killer cells is functional and upregulated after transplantation. *Blood Adv* 2021;5:1069–1080.

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