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Barrett's to Oesophageal Cancer Sequence: A Model of Inflammatory-Driven Upper Gastrointestinal Cancer

Sarah L. Picardo Stephen G. Maher Jacintha N. O'Sullivan
John V. Reynolds

Department of Surgery, Trinity Centre for Health Sciences, St James's Hospital, Dublin, Ireland

Key Words
Inflammation · Microenvironment · Barrett's oesophagus · Oesophageal adenocarcinoma

Abstract
Cancer-related inflammation is considered the 'seventh hallmark of cancer'; many studies show that tumours develop and progress within inflammatory diseases. This review focuses on Barrett's oesophagus, a common condition in which chronic inflammation and resulting alterations in the stroma can lead to carcinogenesis, specifically oesophageal adenocarcinoma. Changes that occur in the tissue microenvironment during development of this disease are discussed. Infiltration of immune cells facilitates tumour development through production of factors that promote carcinogenesis and by enabling tumours to evade the host immune response. Small molecules including cytokines, chemokines and growth factors play key roles in both inflammation and cancer by promoting proliferation, angiogenesis and carcinogenesis and by recruiting immune cells. The extracellular matrix is altered in inflammation, and provides structural support to developing tumours. Hypoxia is a common state in cancers and inflamed tissues which causes DNA damage and induces tumourigenic factors. Finally, tissue vasculature is a vital part of its microenvironment, supplying oxygen, nutrients and growth factors to rapidly dividing cells, and providing a mechanism for metastatic spread. The cells and molecules outlined here represent potential targets for treatment of this cancer, especially in its pre-cancerous, inflammatory stage.

Introduction

The link between the tumour and its environment has been established and is hypothesised to be driven by chronic inflammation, considered now to be the seventh hallmark of cancer [1]. Tumour cells are dependent on their environment for development and progression [2, 3]. The inflammatory response in the tumour microenvironment is characterised by infiltration of immune cells, alterations in cytokines and chemokines, and vascular changes. Chronically inflamed tissues, in which these changes are often already present, are innately susceptible to tumour formation. Barrett's oesophagus (BO) may be an exemplar model of inflammation-associated cancer. It is characterised pathologically by specialised intestinal metaplasia and develops from chronic gastro-oesophageal reflux of acid and bile, and it represents the sole pathological precursor of oesophageal adenocarcinoma (OAC). The Barrett's to cancer spectrum progresses from specialised intestinal metaplasia through low-grade dysplasia (LGD) and high-grade dysplasia (HGD) to OAC. In this review, we describe various aspects of the oesophageal microenvironment which can promote progression to

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John V. Reynolds
Department of Surgery
Trinity Centre for Health Sciences
St James Hospital, Dublin 8 (Ireland)
E-Mail reynoljv@tcd.ie

cancer. First, we examine immune cells that infiltrate the tissue in BO and during the progression to OAC. Second, we look at the cytokines and chemokines produced during this sequence. Third, we explore the role of the extracellular matrix (ECM) and associated proteins in promoting tumour development in the oesophagus. Fourth, hypoxia and oxidative stress in the oesophagus is examined, and how these factors affect disease progression. Finally, we look at angiogenesis and how this process is facilitated and also facilitates oesophageal carcinogenesis.

Barrett's Oesophagus

BO develops in response to chronic reflux of bile and acid from the stomach and duodenum. OAC, which can develop from BO, has increased 5-fold over the last 30 years [4, 5] and has an extremely poor 5-year survival. The risk of developing OAC in a patient with BO is approximately 30 times that of the general population [6]. It is thought that chronic inflammation in BO may drive progression to OAC; the risk of cancer development in BO has been shown to be decreased with the use of aspirin and other anti-inflammatory drugs [7–9], and it is suggested that non-steroidal anti-inflammatory drugs (NSAIDs) may act after the formation of BO but before OAC develops, implicating inflammation as a causative factor in OAC development.

Role of Immune Cells

Immune cells are found in all tumours and inflammatory conditions. They can facilitate tumour development through the production of factors that promote carcinogenesis and by helping the tumour to evade the host response by creating an immunosuppressive environment. Immune cell infiltration is observed during all stages of the progression from BO to dysplasia to OAC, although there is still a lack of significant research into the roles that they play in oesophageal disease. Dendritic cells (DCs) have been shown to be increased in BO tissue compared with normal oesophageal tissue [10]; DCs may also play a role in progression to cancer, as they are found at greater density in OAC than in BO, often in clusters with T and B cells in the lamina propria [11]. Although the role of DCs remains unclear, an intriguing proposal is that DCs in the oesophageal microenvironment may activate dormant stem cells, identified by the stem cell marker Musashi-1, causing the development of BO and OAC [12].

T-helper cells are located in both squamous epithelium and in Barrett's epithelium, while precursor (CD7+) T cells are most frequently located in adenocarcinoma tissue [13]. NF-κB activation, a marker of inflammation, as well as apotosis and caspase activity are observed in such T cells in Barrett's and OAC. Apoptosis in naive T cells was increased in BO, implying that these cells never develop into mature effector T cells. This suggests that the immune response is compromised in malignant transformation. Intratumoural activated CD8+ T-cell infiltration has been shown to correlate with improved disease-free survival in OAC [14]. Increased CD8+ T cells, along with increased FOXP3+ T_{reg} cells, are associated with a lower stage of tumour [15]; dysregulation of the T-cell response therefore seems to be important in OAC progression.

In BO, a predominantly humoral (Th2)-type immune response is seen, with increased plasma cells and mast cells. This contrasts with non-Barrett's with reflux oesophagitis (RO), which is characterised by a Th1-type immune profile which represents a more pro-inflammatory T-cell phenotype [16]. Lymph follicles (segregated areas of T cells, B cells and DCs) were observed in a subset of Barrett's patients; however, these were not found in RO patients. Infiltration of eosinophils has been shown in the mucosa of a subset of BO patients, associated with basal cell hyperplasia [17]. Macrophages, while found in similar number in RO and BO [16], are increased in OAC and produce the angiogenic factor vascular endothelial growth factor (VEGF) [18] and the matrix metalloproteinase MMP-12 [19], the latter of which is increased in BO, although not to the same extent as in OAC.

Role of Cytokines, Chemokines and Growth Factors

Small molecules, including cytokines, chemokines and growth factors, play key roles in the development of both inflammation and cancer, through the direct effects of promoting proliferation, angiogenesis and carcinogenesis as well as through the recruitment of immune cells. BO demonstrates a multitude of immune mediators in its microenvironment.

OAC has an array of small molecules that play a role in its development from BO. In a cohort of OAC patients, IFN-γ, IL-1α, IL-8, IL-21 and IL-23 were found to be associated with a poor prognosis, with a particularly strong association in known Barrett's-derived cancers [20]. IL-6 was increased in transformed Barrett's cell lines (with activated Ras and p53 knock-down) compared with non-transformed lines, along with its regulator STAT3 [21]. These trends have also been demonstrated in tissue samples from BO and cancer [22, 23]. IL-6 is known to

inhibit apoptosis in other cancers [24, 25], providing a possible carcinogenic mechanism.

TGF-β_1 is an anti-inflammatory and tumour-suppressive cytokine under normal conditions, but, in an abnormal microenvironment, can promote tumourigenesis [26]. TGF-β_1 is significantly increased in OAC tissue compared with Barrett's tissue, and is associated with advanced tumour stage [27]. It has been shown to increase migration and invasion in OAC cells by inducing ECM-degrading enzymes, as well as by causing failure of cell-cycle arrest and thereby increasing proliferation [28]. TGF-β_1 may also be involved in epithelial-to-mesenchymal transition in the oesophagus, thought to play a role in carcinogenesis [29]. In the stromal compartment of oesophageal tissue, the TGF-β-related genes TSP-1, POSTN and TMEPAI were found to be dysregulated across the metaplasia-dysplasia-carcinoma sequence, in addition to inflammatory mediators including IL-6 and COX-2 [30]. BO is associated with upregulated COX-2, a key inflammatory molecule implicated in many cancers, and linked with NF-κB activation, and COX-2 is further increased in dysplasia and adenocarcinoma [31–33], with increased COX-2 expression in OAC associated with worse prognosis [34].

The transcription factor NF-κB has been found to be upregulated along the sequence from BO to OAC in tissue samples, along with one of its target molecules, IL-8 [35, 36]. NF-κB can also be activated by the bile acid deoxycholic acid, a component of refluxate, in oesophageal cells, as well as low pH [37–39] IL-8 was upregulated in the proximal segment of BO in patients compared with the distal segment, along with other pro-inflammatory cytokines like IL-1β. However, in the distal segment, where the majority of cancers develop, the anti-inflammatory cytokine IL-10 was increased, and was also higher in dysplastic oesophageal tissue [40]. This suggests that IL-10, and the Th2 immune response, may act as an immune escape mechanism in the development of tumours in Barrett's epithelium.

A novel way in which small molecules may modulate oesophageal neoplastic progression is via proteins secreted by adipose tissue. As obesity is a recognised risk factor for OAC, the effect of adiponectin and ghrelin, two cytokines decreased in obesity, on OAC cells was examined. It was observed that adiponectin increased apoptosis, while ghrelin decreased inflammation by lowering COX-2 and IL-1β production [41]. The reduction in these factors seen in obese patients may drive OAC progression by decreasing apoptosis and promoting inflammation. Visceral (omental) adipose tissue from OAC patients contain a large population of activated CD4+ and CD8+ T lymphocytes, which produces abundant IFN-γ, further implicating adipose tissue in driving the inflammation seen in OAC [42].

Role of the Extracellular Matrix

The ECM is an often-overlooked factor in the tumour microenvironment, comprised of a meshwork of structural proteins including collagen and fibronectin and matricellular proteins, as well as enzymes such as matrix metalloproteinases. The ECM must be modified in order to support an inflammatory-driven tumour with associated growth factors and immune cells.

Matricellular proteins are a unique class of proteins, the importance of which are now being explored. Secreted protein acidic and rich in cysteine (SPARC) is a protein with multiple effects, including counteradhesive and antiproliferative functions, as well as cell cycle modulation and matrix remodelling properties [43]. SPARC is increased in BO and OAC [44], while its expression is higher in the normal oesophagus of Barrett's and cancer patients compared with healthy patients, suggesting that this may be a field effect in the oesophagus [45, 46]. SPARC may be overexpressed in the tumour microenvironment in an attempt to inhibit tumour growth, while tumour cells themselves reduce SPARC. Another matricellular protein, thrombospondin-1 (TSP-1), has antiangiogenic effects and regulates TGF-β_1 [47]. TSP-1 is differentially expressed across the oesophageal carcinogenic sequence [30]. The matricellular protein osteopontin, which promotes metastasis in many tumours, has been found to be upregulated in OAC, along with the MET oncogene [48].

Another major component of the ECM is the matrix metalloproteinase (MMP)/tissue inhibitor of matrix metalloproteinase (TIMP) system, which is involved in turnover and remodelling of the ECM, as well as tissue growth and angiogenesis. In humans, over 23 MMPs have been identified. They are involved in the processes of inflammation and carcinogenesis, as many are produced by inflammatory and stromal cells, and so may have a role in inflammatory-driven cancers. In the oesophagus, MMP-1 has been found to be increased in BO and Barrett's-derived cancers and in OAC cell lines; it also correlates with lymph-node metastasis and poor prognosis [49–51]. Another study found that MMP-1, -3, -7 and -10 along with TIMP-1 were increased along the sequence from BO to OAC [19]. MMP-9 demon-

Table 1. Overview of factors involved in the inflammatory-driven cancer that develops in the oesophagus

Category	Factor	Alteration	Authors
Immune cells	Naive T cells	Increased apoptosis in BO	Berndt et al., 2010
	Macrophages	Similar in RO and BO; produce VEGF in OAC and MMP-12 in BO-OAC	McDonnell et al., 2003; Salmela et al., 2001
	Dendritic cells	Increase in progression from BO-OAC; may activate stem cells	Bobryshev et al., 2009, 2010
Small molecules	NF-κB	Increases in progression from BO-OAC	Abdel-Latif et al., 2004
	IL-6	Increases in progression from BO-OAC, along with regulator STAT3	Dvorakova et al., 2004; Dvorak et al., 2007; Yu et al., 2009
	IL-8	Increased in progression from BO-OAC	O'Riordan et al., 2005; Jenkins et al., 2007
	TGF-β_1	Increased in progression from BO-OAC	Von Rahden et al., 2006
	COX-2	Increased in progression from BO-OAC	Morris et al., 2001
Extracellular matrix	SPARC	Increased in BO-OAC progression	Botelho et al., 2010
	TSP-1	Dysregulated in BO-OAC progression	Saadi et al., 2010
	Osteopontin	Increased in OAC	Miller et al., 2006
	MMP-1	Increased in BO-OAC progression	Grimm et al., 2010; Keld et al., 2010; Murray et al., 1998
	MMP-3	Increased in BO-OAC progression	Clemons et al., 2010; Lagarde et al., 2007
	MMP-7	Increased in BO-OAC progression	Salmela et al., 2001
	MMP-9	Increased in BO-OAC progression; increased in immature vessels in BO	Herszenyi et al., 2007; Auvinen et al., 2002
Hypoxia and oxidative stress	ROS	Increased in BO-OAC, increased with bile acid exposure	Clemons et al., 2007; Zhang et al., 2009; Feagins et al., 2008; Hong et al., 2010
	HIF-1α	Increased in BO compared with normal	Ling et al., 2009
	HIF-2α	Increased in dysplasia and OAC	Griffiths et al., 2007
Angiogenesis	Microvessel density (CD34)	Increased in BO-OAC progression	Couvelard et al., 2000; Mobius et al., 2003; Saad et al., 2005
	VEGF family	VEGF-C increased in BO-OAC, VEGF-A increased in BO	Couvelard et al., 2000; Auvinen et al., 2002

BO = Barrett's oesophagus, RO = reflux oesophagitis, OAC = oesophageal adenocarcinoma.

strates a similar trend [52], suggesting that alterations in these proteinases may be early events in oesophageal carcinogenesis. MMP-1, -3, -7 and -9 have been found to be prognostic biomarkers for oesophageal cancer [53]. MMP-9 is increased in the immature blood vessels seen in BO and therefore may promote angiogenesis in OAC [54], while mean expression of MMP-9 by OAC cells is higher with increasing tumour stage [55]. Leptin, an adipocyte-derived cytokine, stimulates proliferation of oesophageal tumour cells via MMPs, as MMP inhibitors block this proliferation [56], again linking obesity, the tumour microenvironment and OAC development. There has been a clinical trial of an MMP inhibitor in OAC [57], although results were inconclusive. In another link between inflammation and tumourigenesis, TGF-β can induce the ECM-degrading proteinases urokinase-type

plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) in OAC cell lines; production of these molecules correlates with an invasive phenotype in migration and invasion assays [28].

Role of Hypoxia and Oxidative Stress

In inflammation and carcinogenesis, high metabolic demand from rapidly dividing cells causes greater demand for oxygen than the existing vasculature can supply, and hypoxia occurs. Although in normal tissues hypoxia is generally avoided due to healthy physiological regulation, the loss of these regulatory mechanisms and abnormal angiogenesis in tumours means that hypoxia is a common state. Hypoxia can cause significant DNA damage and induce tumourigenic factors including hypoxia-inducible factors 1 and 2 (HIF-1 and -2). Reoxygenation via the inefficient tumour vasculature can also cause significant oxidative stress through the production of reactive oxygen species (ROS) such as nitric oxide (NO) and hydrogen peroxide (H_2O_2).

HIF-1α is increased in BO compared with squamous epithelium, and correlates with the degree of acute and chronic inflammation. Expression was not further increased in dysplasia or OAC, implying that this may be an early change in neoplastic progression and in inflammation [58]. HIF-2α is increased in dysplasia and further increased in OAC, along with hypoxia-associated proteins VEGF and the erythropoietin receptor, while HIF-2α was not expressed in BO tissue, suggesting that this may be a later change in tumour development [59]. P53 is a key tumour suppressor gene induced by hypoxia and by ROS, and regulates cellular apoptosis. In chronic inflammation and in carcinogenesis, p53 is often mutated and dysfunctional, leading to inactivation of its tumour-suppressive function. In the oesophagus, p53 mutational inactivation correlates with progression from BO to OAC in patient samples [60–63], and overexpression of mutated p53 is seen in OAC [64, 65]. In a BO cell line, knocking down p53 and overexpressing the oncogene Ras caused malignant transformation [66]. Bile and acid treatment, which can induce ROS production in the oesophagus [67], increased p53 expression in BO cells initially, but this expression subsequently decreased with malignant transformation [68], suggesting that reflux may play a role in p53 alterations.

Chronic inflammation in the oesophagus producing oxidative stress is thought to increase tumour formation through DNA damage and increased mutational rate. Chronic inflammation in the oesophagus producing ROS is thought to increase tumour formation through DNA damage and increased mutational rate. Genomic instability, as evidenced by sister chromatid exchange, micronuclei and deletion at chromosomal fragile sites, is increased in BO compared with normal squamous epithelium [69, 70], and further increased in cancers [71]. In Barrett's tissue, dysplasia is associated with increased oxidative DNA damage [72]. Bile acids can induce DNA damage via ROS production [73] and NF-κB activation [74–76] (another link to the inflammatory process), with increases in DNA double-strand breaks and intracellular ROS levels [77, 78]. NO can increase invasiveness of dysplastic and cancerous cells via regulation of MMPs and TIMPs [79]. In BO and OAC cell lines, bile acids induce increased ROS and increased cell proliferation in a complex system, possibly by induction of PI-PLCγ2, ERK2 MAP kinase, and NADPH oxidase NOX5-S via the TGR5 receptor [80, 81]. Similar to findings in the colon, antioxidants such as reservatrol and vitamin C can block DCA-induced NF-κB expression [75], while people with high antioxidant intake have a 50% decreased risk of developing OAC compared with those with a low intake [82].

Role of Angiogenesis

The tumour vasculature provides oxygen, nutrients and growth factors to the rapidly dividing cells, and provides a mechanism for metastatic spread. The link between inflammation and angiogenesis has been well described [83, 84]. Endothelial cells in the vasculature facilitate the inflammatory response by modulating the influx of leucocytes via adhesion molecules and chemokines, and by forming new vessels [85, 86]. Expression of VEGF has been demonstrated in almost all cancers. This family of growth factors are potent stimulators of endothelial cell proliferation and migration.

In the oesophagus, microvessel density, as measured by CD34 staining, increases from BO to HGD to intramucosal carcinoma, which correlated with VEGF expression [87, 88]. Angiogenesis and VEGF have also been shown to correlate with lymph node metastases in OAC [89]. VEGF-A is expressed by goblet cells in BO, and its receptor is expressed on new vessels in the tissue [54]. The lymphangiogenic factor VEGF-C was increased from normal to BO to OAC tissue and microvessel density increased similarly along this sequence. Another marker for angiogenesis, endoglin or CD105, a member of the TGF-β_1 receptor complex, increased from LGD to HGD to OAC, while VEGF expression correlated with

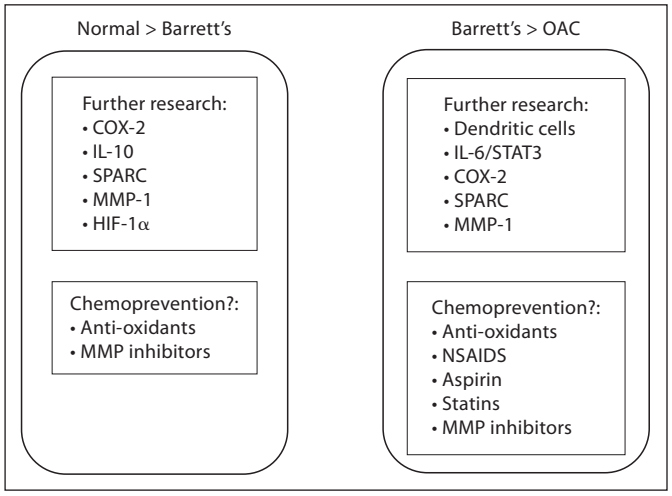

Fig. 1. Overview of potential targets in the oesophageal disease microenvironment which warrant further investigations and/or clinical trials. Further research: these molecules, previously demonstrated to be expressed in the tissue environment, may provide targets for prevention of development or progression of Barrett's oesophagus and oesophageal adenocarcinoma. Chemoprevention: therapeutic agents in this figure should be further investigated as to their potential role in treatment and/or prevention of oesophageal disease, based on previous research.

angiolymphatic invasion [90]. In an interesting link between angiogenesis and inflammation, COX-2 expression in OAC correlates with neovascularisation (CD31 staining) [91] and VEGF expression [92]; this is supported by evidence in other tissues that COX-2 inhibitors can suppress vessel growth [93]. In BO patients, CD34 vessel staining is associated with reflux symptoms and COX-2 expression, suggesting that bile and acid may induce angiogenesis via COX-2 expression [94].

Therapeutic Targets

The microenvironment in BO and oesophageal cancer provides an ideal target for interventions, since it plays a vital role in the development and progression of this tumour. The cells and molecules outlined in this review represent potential targets for treatment of this condition, especially in precancerous, inflammatory BO. The areas of research that may warrant further investigation and trials are outlined in figure 1. BO is often asymptomatic or the symptoms may be easily controlled, and so targeted medical treatment for BO is relatively under-researched, while endoscopic treatment is becoming more readily available and successful [95]. The most commonly prescribed treatment for BO is a proton pump inhibitor, which decreases acid production and therefore acid reflux into the oesophagus. In general, proton pump inhibitors have been found to have little or no effect on Barrett's tissue itself or on molecular markers such as p53 or COX-2, or DNA methylation [96–98]. Aspirin and NSAIDs have been found to have a protective association with the development of OAC, with aspirin having an especially beneficial effect [7]. Despite the role of COX-2 in OAC development, trials of a selective COX-2 inhibitor, celecoxib, found no protective effect on progression of BO [99]. Currently a large-scale randomised controlled trial, the AspECT trial, is underway in the UK, examining the chemopreventative effect of aspirin on OAC [100]. Other targets in the oesophagus include the ROS which are known to play a role in progression to cancer; one study examining dietary antioxidant intake has shown that patients with high antioxidant intake had a reduced risk of OAC (OR 0.57) and especially those with high vitamin C intake (OR 0.37), compared with those with low intake [101].

Concluding Remarks

Inflammation fuels almost every aspect of the carcinogenic process in the Barrett's to OAC paradigm – the production of growth factors, cytokines and chemokines that stimulate tumour cell development, the immune cells that both protect the developing tumour from the host response and also cause the oxidative stress which facilitates DNA damage and cancer development, angiogenesis that provides vasculature needed by the growing tumour, and the ECM needed by the tumour for structural support, invasion and metastasis. BO has an inflammatory origin, and the resulting tissue microenvironment provides the conditions needed for tumourigenesis. Central processes in this disease seem to be the activation of NF-κB and STAT3, as well as neovascularisation, oxidative damage and p53 inactivation due to chronic inflammation. These core changes seem to trigger a cascade of pathways involving various cytokines, especially IL-6 and TGF-β, and other molecules such as the MMPs and VEGF; these pathways facilitate the development and proliferation of cancerous cells and allow them to metastasise. Changes occurring in the inflammatory microenvironment of the oesophagus during disease progression are summarised in table 1 and figure 2.

Although there has been an increase in research into tumour microenvironments, there remains much to do

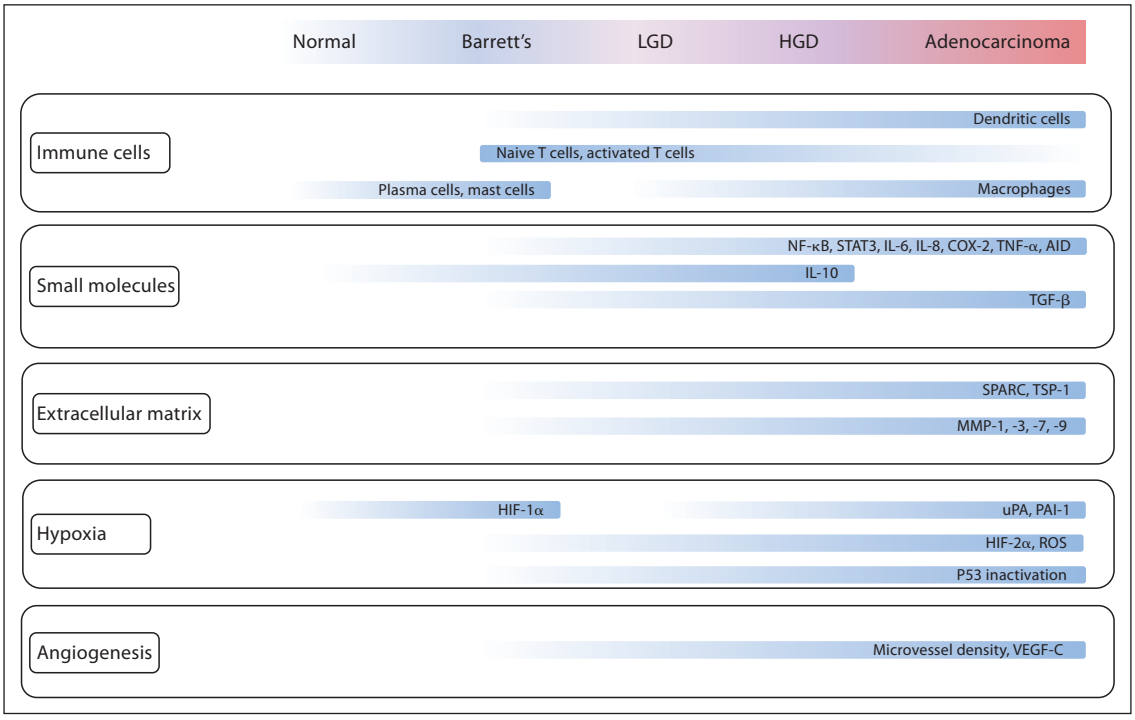

Fig. 2. Overview of factors involved in the inflammatory microenvironment during oesophageal disease progression. This figure outlines the main factors that increase or decrease during the progression of oesophageal tissue from normal squamous epithelium to Barrett's metaplasia to oesophageal adenocarcinoma, categorised by the aspects of the microenvironment described in this review: immune cells, small molecules, ECM, hypoxia and angiogenesis. Bar shading density indicates relative abundance. LGD = Low-grade dysplasia; HGD = high-grade dysplasia.

before aspects of this area can be targeted therapeutically. Ubiquitous transcription factors, such as NF-κB and STAT3, are unlikely targets due to their multitude of downstream effects – more specific molecules, such as cytokines or MMPs, would have fewer side effects if neutralised, and would provide a more direct target. With continued research in this premalignant inflammatory disease, real therapeutic targets may emerge. Directing treatments at the inflammatory microenvironment means that cancer cells never gain the immune infiltrate, vasculature and ECM that they need to develop and survive. In this common condition, prevention of tumour development by targeting the carcinogenic tissue microenvironment is the ultimate aim.

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