Role of the micro-environment in Barrett’s carcinogenesis

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Abstract
Most epithelial cancers occur on the background of chronic exposure to damaging agents which is reflected in the long lag phase from development of a pre-invasive lesion to the development of a carcinoma. Luminal refluxate has long been recognized to be associated with Barrett’s oesophagus, although causal mechanisms have not been clearly defined. Recently, obesity and dietary nitric oxide have also been implicated in the disease pathogenesis. We have demonstrated that acid can alter cell kinetics and, together with nitric oxide, can induce double-stranded DNA breaks. Aside from exposure to luminal factors, the stromal micro-environment may also be important. There is increasing evidence to suggest that inflammatory pathways such as TGF (transforming growth factor) β may play a role in Barrett’s oesophagus carcinogenesis. Hence stromal-epithelial-luminal interactions may influence cell behaviour. As sequelae to this, it is possible that the niches created by the micro-environment may influence genetic epithelial diversity observed within the Barrett’s oesophagus segment.

Introduction
Cancer is a stepwise process in which key genetic abnormalities provide cells with the capability of self renewal, invasion and metastasis. Although a gradual process, evolving over a number of years, there appears to be a relatively small number of key genetic changes which drive disease progression [1]. This has led to the idea that if the key growth factor receptors, upon which the tumour has become dependent by a process known as oncogenic addiction, can be identified and targeted, then the disease process can be halted. However, in Barrett’s oesophagus, the key genetic changes driving the disease have been harder to identify and, although there is a large catalogue of molecular abnormalities characterizing the various disease stages through metaplasia and dysplasia in the published literature [2], the critical causal changes are not well identified in comparison with the bystanders [3]. Recent work in which two clonal markers (p16 and p53) were painstakingly characterized from archival tissue on a gland-by-gland basis has suggested that Barrett’s oesophagus is very heterogeneous [4]. This leads to the possibility that Barrett’s oesophagus is a polyclonal disease. The underlying reasons for that could be genetic instability or alternatively that the diversity of micro-environments create different niches which influence the epithelial genotype and hence the phenotype [5]. The possible micro-environmental influence includes both luminal and stromal factors.

Influence of luminal factors on Barrett’s oesophagus epithelium
Exposure to acid and bile components of refluxate have been known for some time to be associated with the presence of Barrett’s oesophagus [6]. Early work demonstrated that pulsatile exposure to acid-induced proliferation [7,8] and apoptosis [9] in vitro, with some evidence from patient material that the degree of acid suppression may affect the labelling index of Barrett’s oesophagus tissues [10,11]. Apart from altering proliferation, acid has more recently been shown to be capable of inducing phosphorylation of histone H2AX, which is a marker of double-stranded DNA breaks [12]. This seemed to be via the generation of reactive oxygen species, since this effect could be ameliorated by the presence of an antioxidant.

The acidity of the stomach also has a physiological role in the metabolism of dietary nitrate. In the context of reflux disease, the salivary nitrite first encounters acid in the lower oesophagus and this leads to the generation of N-nitroso compounds and nitric oxide [13,14]. Nitric oxide is also capable of inducing double-stranded DNA breaks, but flow cytometric analysis of the cell cycle suggests that this is due to the generation of stalled replication forks rather than being secondary to reactive oxygen species [12].

Prescription of PPIs (proton pump inhibitors) is the recommended first approach to treat gastro-oesophageal reflux disease. However, patients on PPIs have persistence of bile reflux [15]. This is noteworthy since Barrett’s oesophagus epithelium has been shown to express high levels of bile acid transporters [16]. It has been hypothesized that the transporters allow for accumulation of bile acid intracellularly where they activate NF-κB (nuclear factor κB) through the

Key words: acid bile, Barrett’s oesophagus, cytokine, inflammation, luminal factor, stroma.
Abbreviations used: PPI, proton pump inhibitor; TGF, transforming growth factor; TSP1, thrombospondin-1.
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formation of reactive oxygen species [17,18] and promote oxidative DNA damage [19].

**Role of stromal constituents on Barrett’s oesophagus epithelium**

Historically, our knowledge of the molecular mechanisms involved in cancer progression has come from studies of the tumour cell population itself. However, tumours are more than just cancer cells. They comprise a rich cellular and molecular network, including fibroblasts, inflammatory cells, nerve cells, endothelial cells and muscle cells. All of these cell lineages interact via the secretion of a vast array of molecules ranging from the many components of the extracellular matrix, which supports the tissue, to very specific signalling components such as cytokines and growth factors. One can easily imagine the many components of the stroma and any adjacent epithelial as a multifactorial communication network.

Normal epithelial cells depend on physiological support and signalling from the stroma to sustain their functional role and their survival. Whereas cancer cells can modulate their micro-environment through the production of growth factors that affect all of the stromal cell lineages (e.g. fibroblast growth factor and epidermal growth factor), disruption or redirection of normal communication [TGFβ (transforming growth factor β) and cytokines] and reorganization of the extracellular matrix (matrix metalloproteinases), it is only since the 1990s that it has become widely accepted that the stroma plays an integral causative role in carcinogenesis.

Work comparing gene expression profiles of whole biopsies from Barrett’s oesophagus with that of cell lines to subtract a stromal signature suggested that there are similarities in the stroma of the metastatic and cancerous stage [20]. This included expression of a gene, TSP1 (thrombospondin-1), that was not linked previously to Barrett’s oesophagus carcinogenesis. Recently, we have demonstrated, using microdissected stroma from Barrett’s oesophagus biopsy samples, that the stromal gene expression profile alone was sufficient to differentiate between different stages of disease progression [21]. Ontological analyses of these data suggested a preponderance of dysregulation in inflammatory and cytokine pathways in keeping with these data suggested a preponderance of dysregulation stages of disease progression [21]. Ontological analyses profile alone was sufficient to differentiate between different oesophagus biopsy samples, that the stromal gene expression demonstrated, using microdissected stroma from Barrett’s (thrombospondin-1), that was not linked previously to TSP1 stage [20]. This included expression of a gene, similarities in the stroma of the metaplastic and cancerous to subtract a stromal signature suggested that there are biopsies from Barrett’s oesophagus with that of cell lines since the 1990s that it has become widely accepted that the extracellular matrix (matrix metalloproteinases), it is only β growth factor and epidermal growth factor, disruption or cytokines] and reorganization of the extracellular matrix (matrix metalloproteinases), it is only since the 1990s that it has become widely accepted that the stroma plays an integral causative role in carcinogenesis.

In our work, TSP1 was demonstrated to be overexpressed early in the progression to cancer and to be associated with an increased risk of progression with an odds ratio of 3.8 [95% CI (confidence interval) 1.5–9.9, \(P = 0.006\)]. TSP1 has been shown to activate TGFβ and is involved in tissue remodelling [22].

TGFβ is secreted from both the epithelial compartment and the stroma. Manipulations of TGFβ signalling through deletion of TGFβRII (TGFβ type II receptor) in mouse stromal cells, inactivation of Smad4 in T-cells alone or inactivation of BMPRII (bone morphogenetic protein type II receptor) in the stroma alone led to development of prostate and gastrointestinal epithelial tumours [23]. In Barrett’s oesophagus carcinogenesis, Smad-dependent TGFβ signalling is dysregulated early in the progression sequence with lack of responsiveness to the TGFβ anti-proliferative effect in an ex vivo model system. Smad4, which occupies a point in the signalling pathway, was inactivated via a variety of mechanisms including methylation of the promoter [24]. However, TGFβ is a complex growth factor with both tumour-suppressing properties via the Smad-dependent pathway or tumour promotion, via the stimulation of angiogenesis and tumour invasion, depending on the context [25,26]. This phenomenon was also seen in Barrett’s oesophagus carcinogenesis, where TGFβ stimulation of cells unresponsive to TGFβ’s anti-proliferative action up-regulated the extracellular-matrix-degrading protein, uPA (urokinase-type plasminogen activator), and a promoter of cell migration and angiogenesis, PAI (plasminogen activator inhibitor), via stimulation of the MAPK (mitogen-activated protein kinase) pathway [27]. At the pivotal transition point between intraepithelial neoplasia and invasion, it has been shown that TGFβ can promote epithelial–mesenchymal transition [28] and this has been demonstrated for Barrett’s oesophagus and oesophageal adenocarcinoma specifically [29].

**Inflammation**

Inflammation in the distal oesophagus is primarily a response to GORD (gastro-oesophageal reflux disease). Inflammation was first linked to cancer in 1863 following the observation of presence of leucocytes in cancers [30] and has been suggested as the seventh hallmark of cancer [31] to complement Hanahan and Weinberg’s six hallmarks [32]. Over the years, understanding of the role of inflammation has driven the development of novel therapies. NSAIDs (non-steroidal anti-inflammatory drugs), including aspirin, have shown promise as chemopreventive agents for oesophageal adenocarcinoma [33,34], and, in other cancers, specific inhibitors such as IL-6 (interleukin 6) inhibitors are being studied. Although further work is required to understand the precise way in which the stroma can induce cancer in patients with Barrett’s oesophagus, it seems increasingly likely that the stromal compartment, including the recruitment of inflammatory cells in response to reflux injury, are playing a role. A pro-inflammatory Th1 cytokine profile, more characteristic of oesophageitis [35,36], was demonstrated to predispose to Barrett’s oesophagus [37], whereas Barrett’s oesophagus itself was characterized by an anti-inflammatory Th2 response [35,36]. However, it was demonstrated that there was an inflammatory gradient in Barrett’s oesophagus with a pro-inflammatory component in the proximal part of the segment, at the squamo-columnar junction and a more anti-inflammatory component at the distal end [38]. The pro-inflammatory response might be partly due to the more pulsatile exposure to acid and bile of the epithelium and to the cross-talk between the squamous and columnar cells [35,38]. In support of this, the cytokines expressed by co-cultured squamous and columnar cells were more...
representative of a pro-inflammatory response compared with cells grown individually [38]. The inflammatory milieu may influence site-specific cancer development within the Barrett’s oesophagus segment.

Clinical implications

The clinical challenge is to prevent cancer development in patients with Barrett’s oesophagus. In view of the role of reflux components, there has naturally been interest in pharmacological and surgical anti-reflux strategies [39]. There is some evidence for their potential use; however, data from randomized prospective clinical trials are required. There has also been interest in anti-inflammatory medications, and, although epidemiological retrospective data suggest a reduced cancer risk in aspirin users, a prospective trial of celecoxib use failed to demonstrate efficacy. It is also possible that more than one approach is required and the AspECt (Aspirin-Induced Platelet Effect) trial will evaluate the combination of aspirin and proton pump inhibitor therapy (low and high dose) in a two-by-two factorial design. As the specific role of inflammatory and stromal components is unravelled, there may be the potential to use more targeted agents [31].

Technological advances have made ablation of the Barrett’s oesophagus segment an attractive therapeutic option with promising data from a recent randomized controlled trial [40]. The long-term efficacy of such approaches is not yet known, and one key factor that is likely to influence their success is the depth of ablation and the ongoing role of the stromal compartment on any remaining epithelial cells which have the potential to clonally expand.

Conclusions

The current data suggest that the micro-environment can influence epithelial cell behaviour across a number of disease sites, and Barrett’s oesophagus is another example. More research is required to understand the key cell components in the stroma which influence cancer development. Ultimately, such knowledge should enable optimal strategies whether pharmacological, endoscopic or surgical to prevent the progression to invasive adenocarcinoma which has a dismal outcome.

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