Seminar

Barrett's metaplasia

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The rate of oesophageal adenocarcinoma is increasing in the western world and has a poor prognosis mainly because individuals present at a late stage. Attempts to intervene at an early stage of tumour progression have not proven cost effective, although lesions identified during surveillance programmes have a better prognosis. As a consequence, there has been renewed interest in strategies that might prevent the precursor lesion Barrett's oesophagus. Furthermore, there is an improved understanding of genetic and environmental interactions necessary for the clonal expansion and propagation of metaplastic premalignant lesions. Clearly, three mechanisms promote cancer progression—inheritance of germ-line mutations or polymorphisms, sporadic mutagenesis, and local epigenetic alterations. Locally produced cytokines and bile acids in the refluxate create a microenvironment that sets the scene for metaplastic transformation of the oesophageal epithelium, mainly by directly affecting metaplastic stem cells.

Oesophagitis secondary to gastro-oesphageal reflux disease is the most common medical condition in western countries with 30% of adults complaining of heartburn at least once per month, a third of whom will have endoscopic evidence of oesophagitis.[1,2] 40% of patients with oesophagitis improve spontaneously, 50% have persistent oesophagitis, and 10% progress to Barrett's metaplasia.[1,2] Between 0·5 and 2·0% of adults in the western world have Barrett's metaplasia which is a mucin-secreting columnar epithelium that lines the distal oesophagus.[3] Most people develop Barrett's metaplasia in adult life, mainly as a result of duodeno-gastro-oesophageal reflux disease, although any insult that causes distal oesophageal irritation, such as chemical injury, also predisposes to metaplasia. Genetic factors may occasionally play a part in a small proportion of Barrett's metaplasia because it has a familial association and occurs in twins.[2] Oesophageal metaplasia is thought to give rise to most, if not all, oesophageal and gastro-oesophageal junction adenocarcinomas with the rate of neoplastic change each year between 0·2 and 2%. The resulting adenocarcinoma has a uniformly poor prognosis. Once diagnosed, patients have a median survival time of less than 1 year; fewer than 10% of patients survive for more than 5 years despite combined chemotherapy and surgery. The ideal requirement is to detect lesions at an early stage because surgical resection has proven survival benefits.[4]

Conventional clinical risk factors for the development of Barrett's adenocarcinoma are neither sensitive nor specific enough for the classification of individuals at high risk (panel 1).[5–15] Therefore, surveillance of all surgically fit Barrett's metaplasia patients is required, which is neither feasible nor cost effective.[16] Additionally, treatment of both benign metaplasia and the associated cancer is expensive, and attention has centred on understanding basic biology so that novel intervention and therapeutic strategies can be identified.

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There has been renewed interest in primary prevention strategies aimed at prevention of the initiation of Barrett's metaplasia and detection of additional risk factors that more accurately predict the subgroups which will progress to malignant disease (figure 1).[17] Molecular changes in p53, p16, and cyclin D1 overexpression, decreased E-cadherin expression, and loss of heterozygosity of the adenomatosis polyposis coli (*APC*) gene have been detected in dysplastic Barrett's metaplasia and could be used to identify individuals at high risk of developing cancer. These changes could be important because: p53 is associated with the regulation of programmed cell death (apoptosis); p16 and cyclin D1 regulate the cell cycle (especially the G2/M transition phase); E-cadherin is a transmembrane glycoprotein essential for cell-cell adhesion; and *APC* affects intracellular transcriptional protein concentrations such as β-catenin, as well as several other functions. Identification of these alterations remains in clinical research and is not currently used in routine clinical practice.

Two biological issues have been identified in disease progression: the balance between cell proliferation and apoptosis in determining clonal expansion of metaplastic or malignant cells, and the role of altered cell adhesion in remodelling inflamed Barrett's metaplasia. We will discuss the role of environmental factors that might modulate clonal expansion and mucosal remodelling—especially bile acids acting on the apical membrane of metaplastic cells, and cytokines acting on the basolateral membrane.

Oesophageal stem cells

Barrett's metaplasia consists of a simple columnar epithelium that is folded to form glandular invaginations in the mucosa. Cells that are shed from the epithelial surface into the lumen are replaced from below by new cells as a result of stem-cell division. Stem cells can self-renew (clonogenic) and produce indefinite numbers of differentiated progeny. These progeny, termed transit amplifying cells or daughter cells, can undergo a finite number of divisions, but each time they divide they lose some of their capacity for self-renewal (non-clonogenic). Stem cells can be viewed as seed cells whose progeny colonise the entire epithelium. We believe that metaplasia and dysplasia arise from the stem cells of the native oesophagus or adjacent oesophageal glandular tissue. These stem cells are the only permanent residents of the

Panel 1: **Clinical risk factors for Barrett's adenocarcinoma**

	Highest risk	Lowest risk	Refs
Sex	Male	Female	5
Age (years)	>45	<40	6
Length of Barrett's metaplasia (cm)	>8	<3	7
Duodeno-gastro-oesophageal reflux	Severe and frequent*	Mild and infrequent†	8
Chronicity (years)	>10	<1	8
Race	White	Black	9
Body-mass index	Obesity	Normal weight	10
Family history	Gastric cancer	None	11
Drug therapy	Nitrates, benzodiazepines, anticholinergics, theophyllines	Non-steroidal anti-inflammatory drugs	12
H pylori	Absent	Present	13
Smoking	Heavy smokers	Non-smoker	14
Mucosal damage	Ulceration or stricture in Barrett's metaplasia	Intact mucosa	15

*More than three times per week, †less than once per week.

epithelia and are induced to undergo altered differentiation as a result of chronic epithelial damage.[17] The development of metaplasia and progression to adenocarcinoma occurs over a long period, enabling time for successive genetic events to take place.

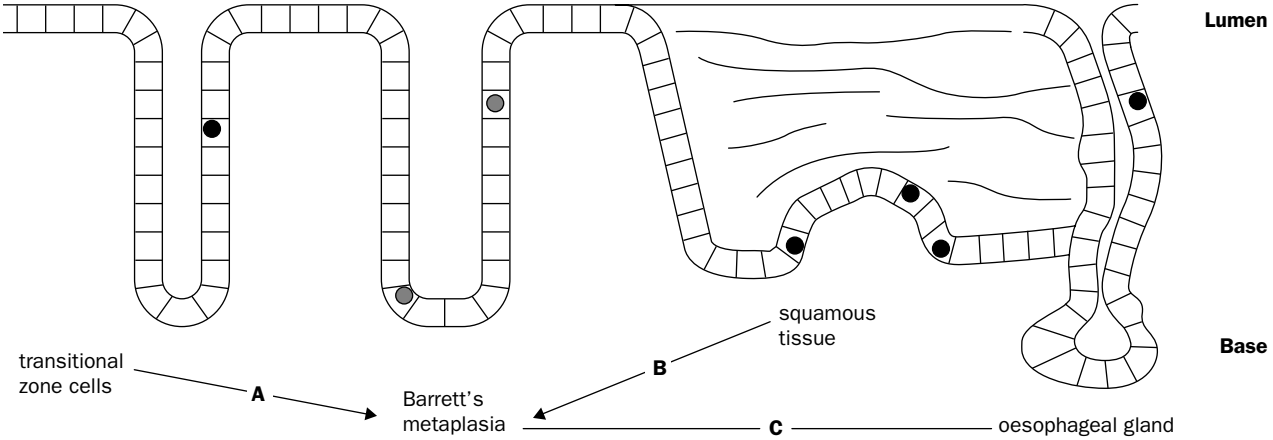
Apart from stem cells most other proliferative gastrointestinal cells are transitory (3–10 day turnover) and are unlikely to play a part in cancer development. Our group has previously shown that squamous stem cells are located in the basal compartment of the normal squamous epithelium, especially at the tips of the papillae. There are, however, no studies identifying the location of stem cells in the metaplastic Barrett's epithelium.

The tissue of origin for Barrett's metaplasia is not clear and three theories exist. First, the de novo metaplasia theory is that the stem cells of inflamed squamous mucosa in the exposed papillae are damaged (figure 2). The resulting phenotypic or metaplastic change in these cells produces Barrett's stem cells. The parallels between the formation of Barrett's metaplasia in squamous epithelium and mucinosis in the squamous vagina lend support to this theory.[18]

Second, the transitional zone metaplasia theory is that cells at the gastro-oesophageal junction (transitional zone) colonise the gastric cardia or distal oesophagus in response to noxious luminal agents (figure 1). As an indicator of the pluripotency of transitional zone cells, Sampliner and colleagues[19] reported that these cells can express a columnar phenotype in the oesophagus and a squamous phenotype in the gastric cardia in response to oesophageal injury. Similarities exist between the structure of the gastro-oesophageal junction and transitional zones in other parts of the body such as the anal canal, uterine cervix, and the prostate gland, where the boundary between two different epithelial tissues is usually highly variable.[20]

Third, the duct-cell metaplasia theory is that stem cells located in the glandular neck region of oesophageal ducts might selectively colonise the oesophagus when squamous mucosal damage occurs (figure 1). The basis for this mechanism of metaplasia is the ulcer-associated cell lineage that occurs adjacent to ulceration in the gastrointestinal tract.[21] A heterogeneic stem-cell response might be predetermined by regional characteristics of the stem cells. For example, cells arising from the transitional zone of the gastro-oesophageal junction might have increased premalignant potential in a similar manner to cells in transitional zones of the cervix and anal canal (panel 2).

Although these differences in stem-cell biology might



● Possible locations of stem cells of the gastro-oesophageal junction: basal zone of the squamous mucosa, necks of oesophageal glands and the transitional zone region

● Possible locations of stem cells in Barrett's metaplasia

Figure 1: **Epithelial compartments from which Barrett's metaplasia arises**

Initial causative stimuli: noxious stimuli resulting in carditis might expand the transitional zone cells (A), or gastro-oesophageal reflux might result in oesophagitis causing damage of squamous stem cells (B), or mucosal ulceration might result in expansion of the ulcer associated cell lineage that arises from oesophageal glands (C). Barrett's mucosa does not usually exist in the oesophagus. Native squamous oesophageal epithelium, oesophageal glands and the transitional zone epithelium are located next to Barrett's metaplasia and are therefore probable tissues of origin.

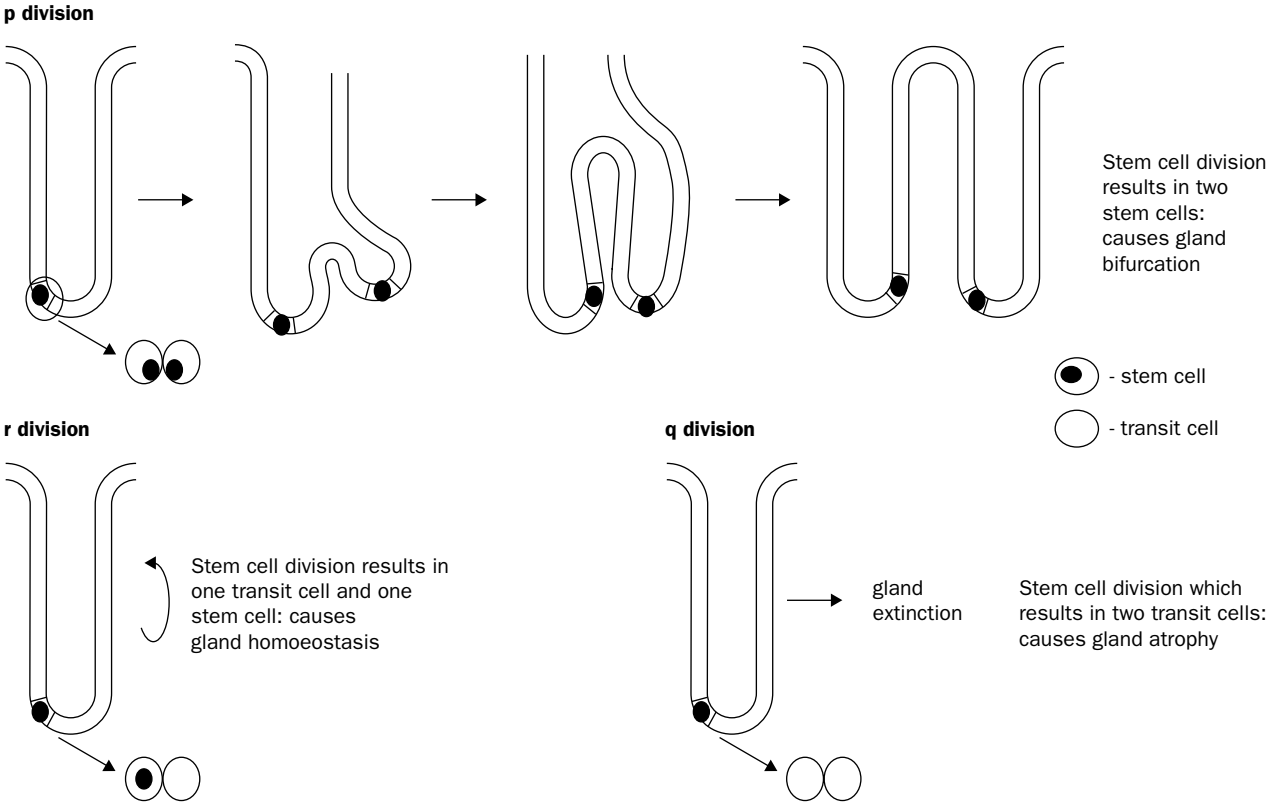


Figure 2: **Role of stem cell number in controlling glandular phenotype**
The fate of stem cells after their division is vital in remodelling the epithelial architecture of Barrett's mucosa. Usually one stem cell will give rise to one stem cell and one transit cell (r division), which will result in tissue homoeostasis. Under other conditions two stem cells (p division) or none (q division) might be produced resulting in gland bifurcation or gland atrophy.

account for regional differences in cancer risk such as increased frequency of adenocarcinoma at the gastro-oesophageal junction, the stem cells require extrinsic factors to initiate and maintain clonal expansion. One important event, which is unknown, is how the initiation of the disease occurs. However, investigators have described some of the extrinsic factors which cause changes in metaplastic stem cells, resulting in colonisation of the epithelium and progression to adenocarcinoma. Two such environmental signals that might modulate this event are cytokines in the inflammatory cell infiltrate and gastric acid and bile acids in the refluxate.

Clonal expansion

After the initial selection or generation of a metaplastic stem cell, clonal expansion takes place, which depends on the control of stem-cell number per gland. The number of stem cells is usually highly controlled so that the production of new cells does not upset homoeostatic balance. However, knowledge of stem-cell biology in the intestine has shown that any abnormality in stem-cell division causes extreme alterations in glandular organisation, structure, and function (figure 2).[22] Usually stem-cell division results in two new cells, one stem cell and one transit cell, which eventually undergoes apoptosis after a series of finite divisions. Alternatively, under special circumstances such as mucosal irritation and ulceration, stem cells can divide to produce two stem cells.

Schmidt and colleagues[21] believe that when the proliferative activity of a gland passes a certain threshold a second, and unknown, independent signal induces the gland to bifurcate in two (starting at the base), termed gland bifurcation. Whereas the production of two functional stem cells is generally associated with glandular bifurcation, the production of two transit cells might result in glandular hyperplasia followed by glandular extinction or atrophy.

Ultimately these bifurcating glands, together with neighbouring identical glands, divide again producing a large contiguous group of epithelial cells with a common genotype, in a similar way to clonal colonisation of the colon by aberrant crypt foci. This large group of epithelial cells is highly variable in different tissues, being about 1 cm^2 in the stomach and colon, and less than 0·3 cm^2 in the small intestine.[23]

The squamous oesophagus has a monoclonal pattern of proliferative organisation, as confirmed by work on glucose-6-phosphate dehydrogenase.[24] However, in the stomach there are regional variations in clonality along a

Panel 2: **Features of three tissues that might give rise to Barrett's metaplasia**

Tissue type	Phenotype change	Malignant risk	Analogous metaplasia
Native oesophageal mucosa	Squamous to columnar	Low	Vaginal mucinosis
Transitional zone cells	Mixed squamous and columnar	High	Cervical metaplasia
Oesophageal gland duct cells	Columnar to columnar	Very low	Ulcer associated cell lineage

In each example of oesophageal tissue another analogy of metaplastic change exists in other regions.

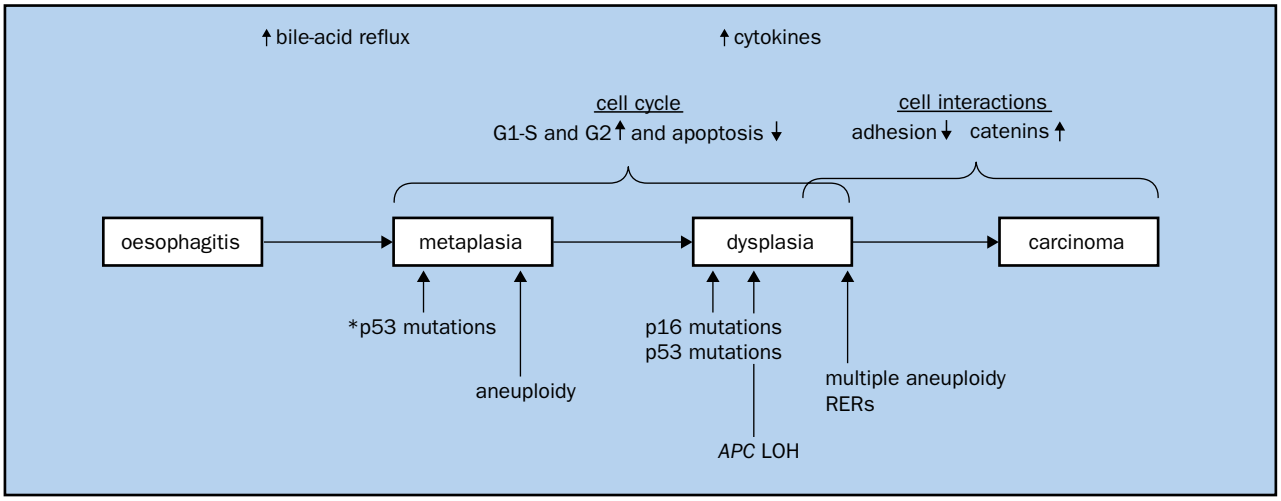


Figure 3: **Apoptosis and adhesion in progression of Barrett's metaplasia**
Progression of oesophageal cells to cancer follows the metaplasia-dysplasia-adenocarcinoma sequence; characteristic alterations in cell survival and adhesion occur. LOH=loss of heterozygosity. RERs=random errors of replication. *APC*=adenomatous polyposis coli gene. *Early p53 mutations unlikely to be important in initial clonal expansion because inhibition of apoptosis (hallmark of p53 dysfunction) is not seen until late dysplasia.

proximal to distal gradient. For example, fundus glands are thought to be polyclonal, whereas in the antrum they are monoclonal.[22] Zonal proliferative variations could also be present along the proximal distal length of Barrett's metaplasia. The reasons for these differences are unknown but might represent the ability of adjacent clones to have a biological advantage that makes them outgrow the other clones. This process usually takes many years. In Barrett's metaplasia, however, once metaplasia is initiated it tends to colonise the mucosa quickly.[25] Usually maximum proximal colonisation takes place within 3 years, with only 5–10% of metaplasias progressing in surface area thereafter.[26]

This rapid colonisation is similar to that seen in other inflamed epithelial tissues such as ulcerative colitis. Rapid colonisation might be due to non-bifurcating mechanisms. In this respect one theory that should be tested is the role of lateral migration of individual stem cells into neighbouring glands, colonising tissue areas by proliferation. This process of lateral migration and rapid clonal expansion has been reported in other epithelia such as the Borst-Jadassohn-type intraepidermal carcinoma in Bowen's disease and urothelial dysplasia.[27] This rapid clonal expansion seems, however, confined to lateral migration of dysplastic tissues, including Barrett's metaplasia rather than the process of gland bifurcation noted in benign metaplastic expansion (J Jankowski, personal observation).

Crypt bifurcation and Barrett's metaplasia have both been reported to occur in the oesophagus of previously unaffected individuals within several months of initiation of chemotherapy or radiotherapy.[28,29] Metaplastic stem cells can respond to exogenous stimuli and seem sensitive to DNA damage, and can undergo apoptosis as a result of p53-mediated mechanisms. The growth of a lesion is determined by a balance between the rate of cell division and the rate of cell death. Therefore, genetic mutations that reduce the probability of apoptosis, such as p53 and p16 mutations, loss of heterozygosity of the adenomatous polyposis gene, or abnormal chromosomal number (aneuploidy), are important aspects of the evolution of a cancer (figure 3).[30] These mutations support the dogma that cancers evolve initially by a series of finite increases in cell population and explains the long lag periods between the initiation and subsequent progression of most cancers.[31]

Role of bile acids

Gastro-oesophageal reflux of acid and bile are the predominant initiating factors in Barrett's metaplasia, although the precise mechanism of cytotoxicity is unclear. Partly regressed metaplastic mucosa might be induced by ablation of acid (and bile) reflux with either proton pump inhibitors or antireflux surgery.[32] However, a series of in-vitro experiments showed that intermittent exposure to acid causes epithelial changes, which could be interpreted as selecting poorly differentiated cells with increased proliferative potential.[32] Additionally, there have been similar findings in patients taking proton pump inhibitors with incomplete acid suppression.[33] Investigators have concluded that the population of patients with Barrett's metaplasia represent a heterogeneous group with a variable response to proton pump inhibition despite symptom control.

At least 35% of patients fail to achieve normal intraoesophageal pH on recommended doses of proton pump inhibitors.[33] Although this treatment is highly effective in healing squamous oesophagitis, it does not convincingly reverse or halt progression of Barrett's adenocarcinoma.[34] Conventional acid-suppressing drugs seem to have succeeded in suppressing symptoms but might have failed to inhibit the evolution of preneoplastic Barrett's metaplasia. In this respect attention has turned to the role of bile acids in the generation of Barrett's metaplasia and its cancer.

Evidence suggests that bile acids alone could be highly important in Barrett's metaplasia, especially since most bile acids are active in the refluxate.[35] First, the development of accurate portable spectrophotometers and sensitive bile salt harvesting methods have shown that reflux of duodenal juice containing bile acids is more common than results of previous investigations suggest.[35] Second, Garewall and colleagues[36] have shown that conjugated bile acids (acidic steroids with detergent properties) might exacerbate oesophageal mucosal injury either alone or in combination with acid, both in vitro and in animals. Third, neoplastic progression of Barrett's metaplasia has been reported in several patients who had bile reflux without any pathological acid reflux.[37] Furthermore, increased oesophageal exposure to total bile acid correlates with worsening mucosal damage, the appearance of Barrett's metaplasia, and especially the

extent of this disease.[38] Fourth, bile acids have also been implicated in the promotion of goblet-cell-containing metaplasia in other gastrointestinal epithelia, including the stomach, duodenum, intestine, bile ducts, and the oesophagus.[39]

Potent acid-suppressing medication might increase the effects of unconjugated bile acids either directly by increasing bile acid transformation or bacterial flora or indirectly by increasing their activity.[36,39] Evidence from animals suggests that acid suppression might increase clonal progression in some patients with bile reflux. The mechanism of bile acid induced progression is complex and involves the induction of cyclo-oxyenase-2 (COX-2) expression which is important in cell survival.[40] Additionally, deoxycholic acid stimulation in vitro causes apoptosis in metaplastic cells expressing wild-type p53, whereas cells with p53 mutations are resistant to bile-acid-induced cell death (J Jankowski, personal observations). All evidence suggests that bile acids may have a much greater role in the progression of Barrett's dysplasia and the development of cancer than hitherto thought. Why does metaplasia not regress once the external stimulus for its initiation is removed? This question can be answered by evidence indicating that a chronic inflammatory cell infiltrate exists in Barrett's metaplasia patients treated by ablation therapy, and may maintain and promote the metaplasia.

Mucosal inflammmation

Mixed inflammatory cell infiltrate is a common feature of acid and bile damage to the native oesophageal mucosa, especially around the stem-cell rich areas of the basal mucosal compartment and papillae.[41,42] This infiltrate is initially composed of acute inflammatory cells. Subsequently T lymphocytes become more numerous, especially in tissues in which metaplastic foci develop.[42] Once duodenal gastro-oesophageal reflux disease is corrected by powerful acid suppressing drugs Barrett's metaplasia never regresses totally and maintains a mild chronic inflammatory infiltrate.[43] After endoscopic ablation therapy, persistent Barrett's metaplasia is associated with a T-cell infiltrate, which is absent in the neosquamous islands;[43] thus lymphocytes might not be a secondary reaction to aberrant epithelial integrity or injury but might be important in the persistence of Barrett's metaplasia.[44]

Associations between inflammation and gastrointestinal metaplasia include helicobacter-positive gastritis with intestinal metaplasia;[45] pancreatic metaplasia with carditis of the gastro-oesophageal junction;[46] pancreatitis with islet cell metaplasia or ductal metaplasia;[47,48] colonic metaplasia with ileal pouches;[49] gastric metaplasia with duodenitis and coeliac disease;[50] diversion colitis with Paneth cell metaplasia;[51] squamous metaplasia with gastric ulceration;[52] ulcerative colitis with metaplastic polyps;[53] and gastric carditis with intestinal metaplasia.[54]

Inflammation due to foreign bodies or parasites can specifically induce goblet cells at the expense of other metaplastic cell lineages.[55,56] The close association between lymphocytic cells and dysplasia suggests that they are necessary for neoplastic progression. The inflammatory cell infiltrate in oesophagitis and Barrett's metaplasia might contribute to DNA damage by the generation of reactive oxygen species (free radicals). High levels of free radicals have been identified in ulcerated gastro-oesophageal mucosa[57] and can induce cytokines, which regulate the extent and phenotype of the infiltrate. Free radicals might also act as, or induce, growth and survival factors for epithelial cells.[58] Moreover, the inflammatory infiltrate can induce increased expression of Fas ligand on metaplastic cells, which might protect from immune surveillance.[59] Furthermore, cytokines can regulate matrix-degrading enzymes such as metalloproteinases, which affect the ability of malignant cells to invade surrounding tissues.[60] Thus, there may be two fundamental stages in the creation of Barrett's metaplasia: (a) mutation of genes by chromosomal or microsatellite instability leading to dysfunction of p53 in severe dysplasia; (b) promotion and propagation of metaplastic clones by an inflammatory cell infiltrate.

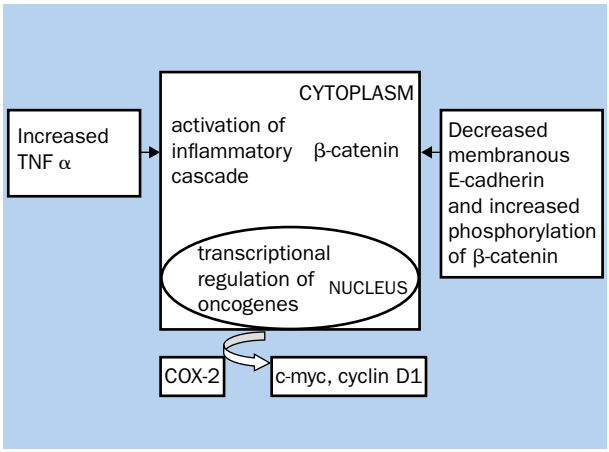


Figure 4: **Microenvironment of the oesophageal stem cell**
The initiation of clonal expansion might take place as a consequence of TNFα secretion from the inflammatory cells and TGFα from adjacent damaged epithelial cells. Both these molecules induce the transcription factor NFκB and catenin-regulated signaling in metaplastic cells, thereby increasing COX-2, c-myc, and cyclin D1 which increase proliferation and decrease apoptosis.

Cytokines induce epithelial proliferation, survival, and migration, and have been implicated in cancer progression in animals as well as correlated with increasingly poorer stages of epithelial cancers in man. Inflammatory cells also produce cytokines such as tumour necrosis factor α, (TNFα), which regulate the extent of proliferation in murine intestine.[61] Furthermore, mice that do not express the gene encoding TNFα (TNFα knockout mice) are protected from epithelial damage and cancer from environmental agents.[62] Many cytokines are produced by the inflammatory cell infiltrate and the epithelium in Barrett's metaplasia,[44] including transforming growth factor β (TGFβ), interleukin one β (IL-1β), interferon γ (IFNγ), and TNFα (R Harrison, personal communication). Work investigating the association between *Helicobacter pylori* and gastric cancer has shown an inherited predisposition to gastric cancer.[44] Infection of the gastric corpus by *H pylori* is related to the development of hypochlorhydria, atrophy, and malignant disease, whereas infection of the antrum is related to the development of peptic ulcer disease. This divergent response cannot be fully accounted for by bacterial virulence alone and evidence suggests that the divergence is related to the host response. Investigators have shown that enhancing polymorphism of IL-1β gene cluster is associated with an increased risk of developing gastric cancer.[63] Patients with such a polymorphism have an enhanced IL-1β secretory response to *H pylori* infection, and one proposal is that increased IL-1β (a suppressor of gastric acid production) allows progression from atrophy to malignant disease.

Analysis of oesophageal cancer has shown that tumour stage and invasiveness are associated with reduced expression of E-cadherin (cell adhesion and tumour

suppressor, figure 3).[64] The role of E-cadherin in oesophageal cancer is lent further support by similar studies, which show that certain cases of familial diffuse gastro-oesophageal cancer are related to germline mutation of the E-cadherin gene that results in loss of expression of the protein on the cell membrane.[65] E-cadherin associates with the multifunctional cytosolic protein β-catenin via membrane adhesion complexes. Cytokines such as IL-β and TNFα may reduce E-cadherin and transiently increase β-catenin-regulated transcription of oncogenes. Transcriptional action of β-catenin relies on it being tyrosine phosphorylated by endogenously produced TGFα in the metaplastic cells[65] and facilitates transformation and survival of abnormal cells. These cells have a survival advantage because of the increased expression of molecules such as COX-2 and cyclin D1 (figure 4).[66,67] These molecules are also implicated in chronic inflammation, cell survival, and epithelial cell growth.

Conclusion

The process of initiation, clonal expansion, and aberrant epithelial biology explains not only why cancer evolution is a multistage process but also why there is a long lag phase between initiation of metaplastic change and cancer development. A combination of aberrant biology, site of origin, and resistance to environmentally induced apoptosis could explain the heterogeneity and malignant potential of metaplastic cells. We suggest that during the initiation phase of Barrett's metaplasia, there is a generation and selection of clones, which are able to resist the adverse effects of duodenal or gastric reflux disease.

Mutations that inhibit bile-acid-induced apoptosis for example, in *p53* will be an advantage to the initiation of dysplastic growth. A striking chronic inflammatory cell infiltrate, expressing IL-1β and TNFα, is associated with persistence of Barrett's metaplasia as well as the development of dysplasia and adenocarcinoma. Epithelial changes resulting in unpredictable neoplastic behaviour may be induced or potentiated as a consequence of interstitial inflammation. There is a molecular basis for implicating chronic inflammation in cancer development. A greater understanding of the molecular changes involved in chronic inflammation might lead to changes in the identification, diagnosis, and management of those at risk of developing malignant disease. The identification of E-cadherin mutations and TNFα polymorphisms might provide a basis on which to offer screening or expert clinical genetic counselling to individuals at high risk with conventional risk factors, including strong family history, and the presence of metaplasia or dysplasia. Finally, clinical specialists have stated that long-term studies are urgently needed to elucidate whether intervention with potent acid-suppressing drugs, anti-inflammatories, or antireflux surgery will prevent progression of established Barrett's metaplasia (H Barr and S Attwood, personal communication).

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