# Review of Barrett's Oesophagus to Oesophageal Adenocarcinoma: New  Prospect

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## ABSTRACT

Today's knowledge on oesophageal adenocarcinoma and its rising incidence has encouraged researcher to illustrate relationship between Barrett's disease and progression to adenocarcinoma. The incidence of this disease has been accelerated sharply in current decades since people life has changed. Studies have been demonstrated that several potential factors including genetical  and environmental factors are involved on Barrett's transformation. Using different detectable biomarkers and techniques have supported early recognition of adenocarcinoma but still have not clarified pathogenesis of Barrett's oesophagus to oesophageal adenocarcinoma. This review summarizes as much as information in relationship with those evidences that have been finalised in different aspects of cellular and molecular pathogenesis of OA. However, current data shows that bacteria might play new role in the pathogenesis of adenocarcinoma.

*Keywords:* Barrett's oesophagus; Oesophageal adenocarcinoma; Incidence

## INTRODUCTION

Barrett's oesophagus (BO)  was a complicated condition associated with pathological and physiological changes in epithelial form and function(1). It  was a pre-malignant disease of oesophageal adenocarcinoma (OA) in which normal oesophageal epithelia  were replaced by columnar epithelia at gastro-oesophgeal junction(2-8). The increasing incidence of BO and OA over the last two decades shows that it  was becoming a major public health concern in developed countries such as the US, UK and north of Iran. BO was identified sporadically in 10% of patients suffering from chronic gastro-oesophageal reflux disease (GORD)(9). Many studies have been carried out with respect to the factors involved in the cellular and molecular processes associated with the replacement of squamous epithelial cells with columnar cells. Caucasian or Hispanic race, lifestyle and male gender are recognised risk factors(5,6,10,11).

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Lifestyle depends on diet and obesity(12), consumption of tobacco and alcohol(12,13), and high levels of nitrosamines in raw fish consumers(14). Population with GORD and duodeno-gastrooesophageal reflux disease (DGORD) are believed to be the main reasons for BO(15). Although GORD or DGORD remain as key determinants, it is recognised that various factors can cause BO including genetic and environmental factors, and bacterial impacts. The question is that whether or not bacteria can play any role in the metaplastic changes of BO. It known that BO creates new microenvironment in which variety of pathogenic and non-pathogenic bacteria are colonised. Subsequently, pathogenic bacteria can take advantages of the changed environmental conditions on BO to induce or initiate neoplastic transformations over the time.

Epidemiologic evidences show that the incidence of OA has risen faster than any other cancers through the Western with 5 to 15% poor prognosis. The prevalence of BO is 0.2-2% of the adult population worldwide(8,16). It increased from 22.6/100,000 in 1987 to 82.6/100,000 in 1998(5). In 1990, the incidence of BO was 376 in unselected sampling from 100,000 people living in Western countries. In a simultaneous study of colorectal cancer in the USA, BO was detected 5.6%-15-25%(5,16). In 1997, endoscopic studies revealed increasing levels of BO from 19/1000 to 40/1000 in 2002, and there has been a 0.5-1% rise in OA. Statistical analysis indicates that the risk of developing OA is 1 in 20 in BO patients, which is 30-125 fold higher than in the general population(7,16). Current data suggest that there has been a greater than six-fold increase in OA in the USA over the past three decades(17).

**Pathogenicity**

The pathology of progression from BO to OA is currently unknown. Several mechanisms and factors have been reported that might involve in this process. It has been found that GORD in patients with chronic reflux symptoms were a progenitor of BO(6,18). This has been studied in animal models, in which high level of acid secretion were induced by repeated histamine injections leads to epithelial metaplasia and then BO. Laboratory based experiments have shown that bile acid exposure on oesophageal cell lines can damage DNA(19). Apart from many other environmental factors such as gastrin, nitric oxide, and the inflammatory response, it has been postulated that the oesophageal microbiota could play an important role in pathological processes associated with OA(20).

Although the cellular mechanisms of oesophageal cancer are unclear, it was possible that those mechanisms of progression in the colorectal adenoma-carcinoma sequence can help to illustrate the transition of BO to OA. This development is usually associated with the metaplasia-dysplasia- adenomacarcinoma sequences. The necessity of amplification depends on several capabilities, which are first growing independently of cells along with having replication abilities; next, having invasive and metastasis potentiality; and finally, loss of cellular adhesion causes to decrease cell to cell inhibition signal and allows tumour invasion(8,16,21) (Figure1).

In BO, cell proliferation and cell cycle abnormalities are seen in phase G1, which was highly sensitive to extracellular modulatory factors, such as CDK and TGFβ(11). Environmental factors that affect cellular processing includes gastro-duodenal reflux components, bile acids, acidic pH and gastrin, and inflammatory responses(15). It was believed that these are part of BO aetiology in stem cells that leads to metaplasia, dysplasia, and malignancy in the oesophageal epithelium. These processes have been classified in two main categories, cellular and molecular factors, and environmental factors.

**Cellular and molecular factors**

The origin of the oesophageal epithelium cell is somatic stem cells, which are self-renewing and produce differentiated progeny cells. Currently, there are four main hypothesises regarding the origin of metaplastic epithelium in BO. First was "de novo metaplasia" theory in which normal oesophageal epithelial cells can act as stem cells to differentiate gastric or intestinal-type epithelium(4,22). Second theory was "transitional zone metaplasia" in which there is migration and colonisation of gastro-oesophageal junction cells in the gastric cardia or distal oesophagus(15,22). Third theory is "duct cell metaplasia" which is existing stem cells in the neck of glandular region of the oesophageal duct migrate to colonise in the injured oesophageal epithelium. Finally, colonisation of bone marrow stem cells in the oesophagus and re-differentiation into metaplastic cells(22). Transition of Barrett's epithelial cell even based on these theories depends of cellular and molecular changes occur during movement. However, it has been generally accepted that the change from metaplasia to dysplasia is highly complex with inducing and activation of hallmarkers via various genes. These genes underlie changes in cell cycling, intracellular adhesion, and nuclear translocation as part
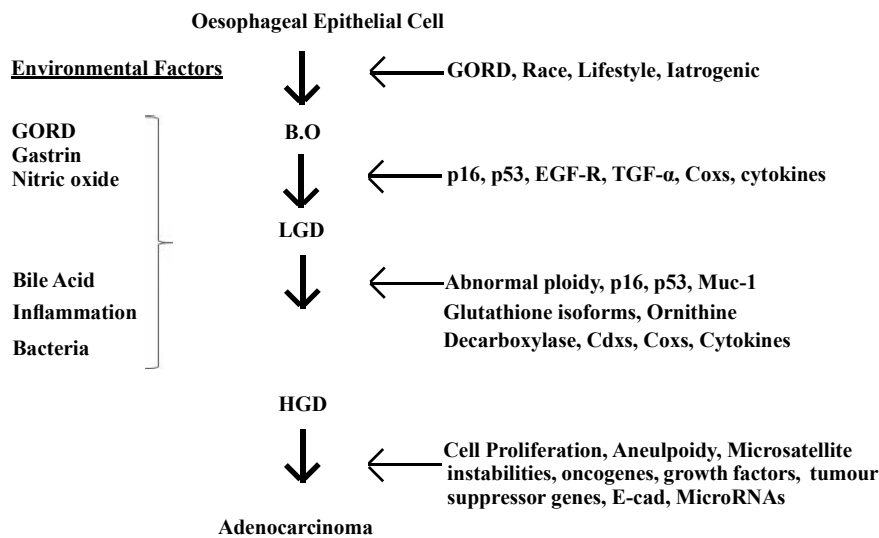
Oesophageal Epithelial Cell

**Environmental Factors** ← GORD, Race, Lifestyle, Iatrogenic

↓

**GORD**
**Gastrin**
**Nitric oxide**

B.O

↓ ← p16, p53, EGF-R, TGF-α, Coxs, cytokines

LGD

↓ ← Abnormal ploidy, p16, p53, Muc-1
Glutathione isoforms, Ornithine
Decarboxylase, Cdxs, Coxs, Cytokines

**Bile Acid**
**Inflammation**
**Bacteria**

HGD

↓ ← Cell Proliferation, Aneulpoidy, Microsatellite
instabilities, oncogenes, growth factors, tumour
suppressor genes, E-cad, MicroRNAs

Adenocarcinoma

**Fig. 1:** Molecular and environmental factors on progression of Barrett's disease

of the cellular and molecular mechanisms (Table 1).

## Proto-oncogenes

Proto-oncogenes are regulatory genes that control cell function by transduction and transcription. Any changes and mutation of these genes can lead to carcinogenesis.

### Ras

Ras oncogenes located on p21 involve H, K and N genes encoding proteins necessary for cell division and differentiation. They operate as signal-transducing molecules in the plasma membrane of the G phase proteins. Immunochemical studies have found variable extensions of ras family proteins that were not coefficient for Barrett's studies in comparison with other carcinomas(2,11, 23).

### Src

There are two different types of src oncogenes, cellular (c-src) and viral (v-src), which encode non-receptor tyrosine kinase to induce signal transduction pathways in cell division, recovery from oxidative stress, and cytoskeletal rearrangements. Mutations in c-src lead to deregulation of cell adhesion and anchorage-dependent growth control to keep cells in the proliferation stage. Immunohistochemical studies have revealed up regulation of c-src in BO and OA(3,23).

### ErbB-2 (HER2/neu)

ErbB-2, located on chromosome 17q21 and has similarity to EGFR, encodes proteins for cell surface receptors of epidermal growth factors. These proteins are a shortened edition of EGF-R in which the EGF-binding domain has been deleted. This results in kinase receptors that cannot bind to EG and remain as active protein-tyrosine kinase. The erbB-2 oncogene has been reported in dysplastic BO(2,11). Its overexpression is related to tumour invasion, lymph node involvement, distant metastasis, and status of residual tumour after resection(23).

## P16

This gene is located on chromosome 9p at the 9p21 locus, with MTS1 and CDKN2AN genes encoding a 16 kD protein that makes a complex with cyclin-dependent kinase 4, 6 (CDK4, 6) to phosphorylate retinoblastoma protein. Inhibition of phosphorylation could block cells entering S phase, and creates uncontrolled cell growth. P16 is a valuable biomarker for the progress of BO to cancer(3). Near to 80% of patients with BO have p16 disorders such as hypermethylation of the promoter, loss of heterozygosity, and mutation. It has been reported that hypermethylation of p16 is indicative of the degree of dysplasia in specialised intestinal metaplasia(2,6,7,23).

This describes changed cells with abnormal chromosomes that can lead to uncontrolled cell division. Flow cytometry, a useful diagnostic technique in the early stages of BO, showed that abnormal DNA contents are seen frequently in G2-M phase, through the sequence of metaplasia-dysplasia-adenocarcinoma.

Therefore, the advantages of aneulpoidy are not sufficiently rigorous to use in histopathological assessments(7,17).

**Telomerase**

Telomerase is a complex of ribonucleoprotein located at the end of chromosomes synthesising telomeric DNA. It is usually a long fragment of non-coding DNA repetition, which preserves the end of the chromosome to degrade an aberrant fuse. Reactivation telomerase enzyme, a ribonucleoprotein reverse transcription that stabilises the telomere, and maintains the proliferative capacity of cancer cells, is seen in cancer cells(24). Increasing activation of telomerase shows preneoplastic condition or neoplastic which leads to genetic mutation. Studies have shown that there is 70% expression of telomerase RNA (hTR) in BO, LGD, and 100% in HGD and OA(17,24).

**MicroRNA**

MicroRNAs, reported first time on 1993 as a novel molecular technique, are small number of non-coding RNAs that regulate expression of genes by post-transcription which leads transcript degradation or inhibition of protein synthesis(25). They intervene with cell differentiation, proliferation, and apoptosis. It is taught that miRNAs might participate in oncogene or tumour suppressor genes processes(26,27) such as lung, breast, prostate, and oesophageal cancer(28). An investigation on miRNA changes between BO and OA showed that there are 44 miRNAs gene expression alternation, in which 5 miRNAs are upregulated in columnar tissue rather than normal squamous epithelium and 3 miRNAs were expressed more in OA than BO(26,29,30).

**Tumour suppressor genes**

Tumor suppressor genes prevent the uncontrolled growth of cells that may result in cancerous tumors.

*P53*

There are regular changes along with metaplasia-dysplasia-adenocarcinoma sequence, which belong to genetic and epigenetic variations. Some genetic variations are observed in cell proliferation and apoptosis and in the tumour suppressor gene. P53 is located on chromosome 17p, and encodes a protein that regulates gene expression, DNA repair, cell proliferation by blocking G0 and G1 phases of cell cycle, and influences apoptosis on the Bax and PIG3 reporter pathways. This is an accumulation of aneulpoidy and abnormality in chromosomal contents, which generates genetic instability and risk of neoplastic progression from LGD to HGD and adenocarcinoma(2,3,6,7,26). The p53 gene product initially is inactive, but is activated by post-translational modifications, such as phosphorylation and cellular stress or damage. These reactions cause increasing p53 expression that restrains cellular division and/or induces apoptosis(31). Mutated proteins of p53 have been reported in BO and OA which occurs in early stage of BO progression in dysplasia. (16,23).

*P63*

Metaplasia is combination of gene expression changes in normal cell function, which occurs during embryogenesis by master switch gene. (16) Differentiation of tissue type is regulated by transcription factors in embryogenesis, encoded by master switch gene (p63) to transfer squamous epithelial cells to the columnar type. P63 is a member of the p53 transcription family factors, and its role has been studied in the development of oesophageal and tracheobronchial epithelia. By knock outing this gene in animal models, it showed that there was no basal progenitor cell to form stratified squamous epithelia, and by over-expression verified its involvement in changing simple lung epithelium to a stratified type(2,8,32).

**Growth factors**

During the sequence of metaplasia-dysplasia-adenocarcinoma, there are various growth factors and their receptors such as epidermal growth factor (EGF) and transferring growth factor-α (TGF-α) that stimulate cell proliferation(3,7,23,33). EGF-R (EGF-receptor) located in chromosome 7p12-13, and TGF-α in 2p13. They are induced throughout the tumour progression and lymphatic dissemination in OA(11). EGF expresses in BO and OA, while expression of EGF-R depends on the degree of dysplasia, in which over expression of EGF-R reflects progression of malignancy. The function of TGF-α depends on EGF and is expressed through Barrett's metaplasia. EGF, TGF-α, and EGF-R are key factors involved in the progression of BO to OA(8,17). Hepatocyte growth factor (HGF-R) is another determinant that is over expressed in 100% of HGD and OA(34). It is reported that patients with negative EGF-R in oesophageal tumours have high surgical resection improvements after 6-month in contrast to those were in the EGF-R positive group(35).

## Cell adhesion molecules

It is believed that cell to cell adhesion molecules (CAM), such as cadherin glycoproteins that hold cells together and mediate cell to cell interactions, play a role in dissemination of cancer(36). In the most epithelia, E-CAD expresses in adhering cells with catenin proteins (β-catenin) and placental-cadherin (P-CAD) in the basal layer of stratified epithelia. Studies have shown remarkable cutting in E-CAD in compare with P-CAD in metaplasia- dysplasia- adenocarcinoma sequences; however, P-CAD was observed in 17 out of 24 carcinomas(37). It has been found that complexes of catenin-cadherin with APC undergo the sequence of metaplasia-dysplasia-adenocarcinoma. Intracellular regulation of catenin within epithelial cells and free cytosolic catenin create degrading catenin components, by which catenin is phosphorylated and produced free in cytosolic form. Consequently, the combination of these components with Tcf/LEF transcription factor enhances the activities of target genes (c-myc, c-jun, cyclin D1, and fra-1). Phosphorylation of tyrosine in β and γ-catenin, which occurs in BO by TNF-α, has been studied in cell culture to phosphorylate catenin and bind cadherin. The results revealed that accumulation of catenin in the nucleus and down-regulation of adhesion result in rising levels of oncogenic transcription. The loss of cell attachment and the destruction of cellular matrices by matrix metalloproteinases (MMPs) has been detected in oesophageal cancer, in which EGF acts as precursors of tumour development to activate MMPs(17). Cyclin D1 (CD1) which regulates proteins in the G1-S phase participates in mitogenic and differentiation signalling pathways. Up to 46%-64% of CD1 over-expression has been defined in BO and OA, and this has been proposed as a diagnostic marker of developing malignancy(2,7).

## Adenomatous polyposis coli (APC)

APC is a loss of heterozygous genes on chromosome 5q21 to 22, and are found on locus 5q of HGD in BO and adenocarcinoma. Produced protein by this gene plays a critical role in several cellular processes that determine whether a cell may develop into a tumour. The APC protein participates in cell division, cell attachment, and cell movement(7,23). This gene participates with catenins to bind transmembranous cadherins to actin filaments of cellular cykoloskeleton(11).

## Caudal related homeobox (Cdx)

Cdx is a common nucleotide sequence in master regulatory genes located on chromosome 13, which regulates the development of animal, fungal and plant genes. Cdx has three types of caudal homologues, Cdx1, Cdx2, and Cdx4, of which only Cdx2 encoding transcription factors that contribute in mammalian homologues of the Drosophila gene caudal. Caudal is a Drosophila homeobox gene that plays a role in primer generating of posterior segment and the final developmental stages of the hindgut(2,38-40). It is known that intestinal differentiation and development depends on transcription factors encoded from these genes. Most investigations have been made regarding the effects of Cdx2 and Cdx1 gene expression in intestinal and oesophageal metaplasia. It is reported that high level of Cdx2 expression induced intestinal metaplasia in a transgenic mouse model. In this model, ectopic expression of Cdx1 generated all four differentiated intestinal cell types, consisting of enterocytes, goblet cells, paneth cells and enteroendocrine cells. Various bile acids, tumour necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) exposure in oesophageal epithelial cells leads to increased Cdx1 and Cdx2 expression. Therefore, it has been established that there is a relationship between Cdxs genes and BO, but molecular framework is still unclear(3,38,41,42).

## Prostaglandins

Synthesis of prostaglandin is regulated by cyclooxygenases (COXs), membrane-associated proteins, which act as catalysers of the process. There are two different types of COXs, Cox1 and Cox2. Cox1 is an essential agent in the gastric mucosa, while Cox2 is usually expressed during inflammation or mitogenic stimulation, and tumour development(5,26,43,44). COXs induce angiogenesis and are involved in the inhibition of immune surveillance, reduction of apoptosis, cell adhesion, and increasing cell proliferation. Invasion, metastasis and attachment to the nuclear peroxisome proliferative activator receptor in the carcinogenesis pathway are also affected by COXs(23). Respectively, in 70% to 80% of BO and OA is seen. Cox2 is expressed in chronic oesophagitis due to bile acid challenge in oesophageal cells. This was tested by inhibition of Cox2 in oesophageal cancer cell lines, in which apoptotic cell death, proliferation activity, and prostaglandin E2 synthesis were observed(5,26,43,44). The latest studies show that there is a gradual enhancement in the expression of Cox-2 throughout the sequence of BO to malignancy, reduction of Cdx-2 expression, and increasing in CDC2, which is a catalytic subunit of protein kinase

**Table 1:** Published evidences of selected studies investigating genetic and epigenetic changes associated with the metaplasia-dysplasia-adenocarcinoma sequence of Barrett's oesophagus

| Markers | | Findings |
|---|---|---|
| Growth self-sufficiency | Cyclin D1 | ↑ nuclear cyclin D1 immunostaining in 46% BE specimens: ↑ cyclin D1 overexpression early event in MDC sequence |
| | | ↑ nuclear cyclin D1 immunostaining in 64% OA specimens |
| | | Cyclin D1 expression correlates with degree of dysplasia in BO |
| | | Cyclin D1 expression 43% BO mucosa (vs 0% normal mucosa) |
| | | Polyphenon E inhibits growth of BO and OA cells via downregulation of cyclin D1 expression |
| | Cyclin E | ↑ cyclin E expression in neoplastic cells in BO |
| | | Cyclin E expression 37% BO mucosa (vs 0% normal mucosa) |
| | p27Kip-1 | 83% OA specimens displayed low p27 protein levels (despite high p27 mRNA): -p27 inactivated in most BO-associated OA (post-transcriptional modification)→loss of cell cycle inhibition |
| | | Experimentally-induced BO and OA development in mouse model significantly enhanced by p27 gene knockout |
| | EGF (and EGF-R) | ↑ EGF in cytoplasm of BO epithelial cells (vs gastric mucosa) |
| | | EGF-R expression area in inflamed mucosa (43.1%) significantly > normal mucosa (29.5%); all BO showed positive EGF-R staining |
| | | EGF/EGF-R expression significantly ↑ in BO and OA mucosa (vs normal mucosa) by flow cytometry |
| | | EGF-R expression positive in 64% of BO-associated OA; ↑ staining associated with poorer survival |
| | | EGF A61G G/G genotype associated with >double OA risk in BO pts (vs A/A or A/G) (OR 2.2) |
| | TGF-α | ↑ TGF-α expression in cells from BO and OA mucosa (vs normal gastric mucosa) by flow cytometry |
| | | TGF-α expression positive in 100% of BO-associated OA |
| | HGF (and HGF-R) | HGF expression significantly ↑ in BO specimens (vs normal esophageal mucosa) |
| | | Intense HGF-R immunostaining in 100% OA and dysplastic BO specimens (vs minimal staining in non-dysplastic BO or normal mucosa); HGF-R mRNA and protein levels ↑ in OA cell lines |
| Erb family tyrosine | Kinases | Membranous c-erbB2 overexpressed in 26% OA (vs 0% BO with dysplasia): -?later event in MDC sequence |
| | | c-erbB-2 gene amplification in 14% OA vs 11% HG-dysplasia vs 0% metaplasia/LG-dysplasia specimens |
| | FGF | Immunostaining intensity for FGF sequentially ↑ from metaplasia/LG-dysplasia (negligible)→HGdysplasia(weak/moderate)→OA (moderate/strong) |
| | | FGF-1 mRNA and protein expression sequentially ↑ in HG-dysplasia/OA (vs metaplasia/LGdysplasia/ controls) |
| Src family tyrosine | Kinases | Src-specific activity 3-4-fold ↑ in BO and 6-fold ↑ in OA (vs controls): -?Src activation early event in MDC sequence |
| | | Strong Src expression in 85% OA vs 93% BO HG-dysplasia vs 72% BO LG-dysplasia vs 27% BO specimens |
| Insensitivity to anti-growth signals | p16 | 9p21 (p16) LOH observed in 89% OA specimens (vs 0% non-dysplastic BO); homozygous p16 deletion in only 25% |
| | | p16 promoter hypermethylation (inactivation) in 75% BO with HG-dysplasia vs 56% LG-dysplasia (vs 3% non-dysplastic BO) |
| | APC | 5q (APC) LOH seen in 80% OA specimens (and surrounding mucosa)] |
| | | APC gene LOH observed in 60% OA specimens (vs 0% non-dysplastic BO) |
| | | APC promoter hypermethylation in 92% OA vs 40% BO (vs 0% normal esophageal tissues) |

| Markers | | Findings |
|---|---|---|
| Avoidance of apoptosis | p53 | Positive p53 immunostaining in 87% OA vs 55% BO with HG-dysplasia vs 9% LG-dysplasia vs 0% non-dysplastic BO |
| | | 17p (p53) LOH found in 91% BO pts who developed aneuploid cell populations: -17p allelic losses precede aneuploidy |
| | | p53 overexpression in 64% OA vs 31% dysplastic BO vs 0% non-dysplastic BO; trend of ↑ p53 expression with ↑tumour grade: -?p53 mutation early event in malignant progression |
| | | p53 immunoreactivity only in OA/BO with HG-dysplasia (not in BO with LG-/no dysplasia); mutated p53 in 69%: -?late event in MDC sequence (during transition to HG-dysplasia) |
| | | p53 protein expression in 85% OA specimens vs 60% BO with HG-dysplasia vs 7% LG-dysplasia |
| | | p53 mutations identified in 75% OA specimens; p53 overexpression in 58% OA vs 60% BO with HG-dysplasia vs 12% LG-dysplasia vs 0% non-dysplastic BO |
| | Fas (CD95) | ↓ surface expression of Fas observed in OA specimens; impaired translocation of Fas to membrane |
| | | wild-type Fas protein retained in cytoplasm in OA cell line: -?potential mechanism by which OA cells evade Fas-mediated apoptosis |
| | | ↓ surface expression of Fas and resistance to Fas-mediated apoptosis observed in OA cell lines |
| | Bcl-xl/Bax/Bcl-2 | Bcl-xl positive in all dysplasia and OA cells, but negative in 47% non-dysplastic BO: -?switch to anti-apoptotic phenotype in transformation from metaplasia to OA |
| | | Bcl-2 expression in 84% LG-dysplasia vs 0% HG-dysplasia or OA |
| | | Cytoplasmic Bcl-xl immunostaining in 59% OA vs 71% BO/HG-dysplasia vs 60% LG-dysplasia vs 27% non-dysplastic |
| | COX-2 | ↑ COX-2 mRNA levels in 80% BO and 100% OA specimens (vs normal gastric controls) |
| | | COX-2 immunostaining strongly positive in 100% BO samples (> gastric controls) |
| | | COX-2 immunopositivity in 91% non-dysplastic BO vs 94% dysplastic vs 97% OA |
| | | Natural/synthetic COX-2 inhibitors suppressed proliferation, induced apoptosis and blocked cell cycle in OA cell lines |
| | | Cox-2 mRNA strongly upregulated in experimentally-induced BO epithelium in rat model (vs absent in control animals); COX-2 overexpression observed in human BO patients with dysplasia |
| Limitless replicative potential | Telomerase | Telomerase RNA positive in 100% OA/BO with HG-dysplasia vs 90% LG-dysplasia vs 70% nondysplastic BO: marked ↑ telomerase RNA accompanies transition along MDC sequence |
| | | human telomerase reverse transcriptase (catalytic subunit of telomerase) expression ↑ at all stages of BO vs normal controls, and in OA and dysplastic BO vs non-dysplastic BO |
| | | Telomerase activity (by telomeric repeat amplification protocol assay) ↑ in OA samples vs adjacent mucosa and in OA vs BO; no difference BO vs adjacent mucosa |
| | | Telomerase inhibition (by small interference RNAs) induced senescence in 40% and apoptosis in 86% in BO cell lines |
| Sustained angio-genesis | VEGF (and VEGF-R) | VEGF expression correlated with higher vascularisation in BO and OA specimens |
| | | VEGF-A expressed in BO epithelium; VEGFR-2 strongly expressed in immature endothelial cells feeding BO epithelium; ↑ VEGF-C expression in BO (vs absent in normal epithelium); ↑ VEGFR-3 in |
| | | OA: ?aberrant neovasculature early in MDC sequence |
| | | VEGF expressed in 64% OA specimens; significantly correlated with angiolymphatic invasion/ survival |
| | | VEGF expression significantly ↑ in OA (> dysplastic BO > BO > normal epithelium) |

| Markers | | Findings |
|---|---|---|
| Invasive/metastatic potential | CAMs | ↓ expression in OA specimens of E-cadherin (in 74%), α-catenin (60%) and β-catenin (72%) |
| | | Abnormal expression of β-catenin, α-catenin and E-cadherin |
| | | significantly associated with higher degrees of BO-related dysplasia |
| | | ↓ expression of E-cadherin with progression along MDC sequence; in contrast P-cadherin |
| | | absent from BO (± dysplasia) but expressed in 67% OA specimens |
| | | Slug (E-cadherin repressor) immunostaining and mRNA levels overexpressed in OA vs BO |
| | | metaplasia specimens: -?Slug upregulation represents mechanism of E-cadherin silencing |
| | Cathepsins | Detected amplicon at chromosome 8p22-23 resulting in cathepsin B overexpression (observed in 73% OA samples) |
| | | ↑ cathepsin C expression in OA (vs BO vs normal) in rat model |
| | CD44 | Stepwise ↑ cathepsin D mRNA levels in GERD→BO→OA tissue |
| | | CD44-H and -V6 variant frequently expressed in BO; differing expression patterns along spectrum |
| | | normal→dysplastic BO→OA: -?CD44H and V6 involved in carcinogenesis of BO mucosa |
| | | ↓ CD44 expression in OA/HG-dysplasia (vs BO/LG-dysplasia) |

MDC: Metaplasia-dysplasia-carcinoma; EGF: Epidermal growth factor; EGF-R: EGF receptor; pts: Patients; OR: Odds ratio; TGF: Transforming growth factor; HGF: Hepatocyte growth factor; HGF-R: HGF receptor; mRNA: Messenger RNA; FGF: Fibroblast growth factor; HG: High grade; LG: Low grade; LOH: Loss of heterozygosity; APC: Adenomatous polyposis coli; VEGF: Vascular endothelial growth factor; VEGF-R: VEGF receptor; CAM: Cell adhesion molecule; GORD: Gastro-oesophageal reflux disease (64).

complex inducing mitosis starting(2,7,43).

### Environmental factors

In this category, characteristics of factors that can be effective will be discussed although alcohol, smoking, and life style might also to be considered, their effects are not as much as selected hallmarks. The important studied environmental factors are as below.

### Gastro-oesophageal-reflux disease (GORD)

It is believed that a variety of factors are responsible for GORD including transient relaxation of the lower oesophageal sphincter, reduction in resting tone of the lower oesophageal sphincter, weakened oesophageal clearance, diminished salivation, bile acid regurgitation from the duodenum into stomach, hiatus hernia, and delayed gastric emptying(4,17). Studies showed that GORD is not only a primary risk factor of BO, it also has 6-10 fold potentiation effect on disease promotion(45). Findings show that GORD can affect the development of BO by influencing CDX gene expression. This is possible by stimulation of oesophageal epithelial cells via gastric reflux components, or by expression of CDX by inflammation of oesophageal epithelial cells. These mechanisms occur by increasing the permeability of epithelial cell, which comes from acid-peptic damage to the tight junction in squamous epithelial cells. Consequently, penetrated components might induce basal epithelial stem cells to express CDX(17,46). Components of duodeno-gastric oesophageal reflux and trypsin also influence BO progression at different pH amounts. It is reported that taurine-conjugated bile salts and complexes of taurodeoxycholate and taurocholate damage oesophageal mucosa at pH2, which is also severely injured by unconjugated bile salts at pH 5-8(47).

### Bile acids

Although the effect of reflux components is still controversial, it is accepted that refluxate composition (bile/acid) has significant role in the progression of BO and AO. The use of long-term acid reduction components such as histamine-2 receptor antagonists, and proton pump inhibitors (PPI) enhanced the risk of developing metaplasia and cancer(11,27). It therefore seems that acid reduction therapy does not prevent acid and bile salt reflux. The DNA destructive effect of bile salts and acid, and GORD on oesophageal cell line has also been studied. Competition between lithocholic acid and retinoic acid in sequestering X receptor is a possible mechanism whereby bile salts can induce BO. Retinoic acid is involved in cell differentiation and development, and founds in high levels in BO(48). Another possible role of bile salts and gastric acid in

BO is in the induction of CDX genes. Various in vitro studies supported that the notion that expression of CDX genes is enhanced by the presence of biliary components, such as mRNA expression of CDX1 in colon cancer cells and induction of CDX2 promoter activity by dehydroxycholic acid and cholic acid(11, 27,49). Dexoycholate and gastric acid increase CDX2 expression by binding transcription factors to Cdx2 promoter in the OA cell line OE19. In HET-1A (human oesophageal cell line) and SEG-1 (Barrett's oesophagus adenocarcinoma cell line) dimethylation of the CDX2 promoter by bile salts and gastric acid result in CDX2 expression. Additionally, it has been shown that by increasing the expression of CDX2 in HET-1A, cells start to form crypt-like structures, and block up-regulation of intestinal genes. Moreover, studies with four human cell lines (HET-1A, SEG-1, HKESC-1, HKESC-2) revealed that bile acids up-regulate simultaneously CDX2 and MUC2 (Mucin2 gene that is expressed by goblet cells) expression in Barrett's epithelium, while there is no MUC2 expression in normal oesophageal epithelia(27,46, 49). Therefore, these findings indicate that bile acids and GORD influence development of BO by induction of CDX gene expression.

### Gastrin

Gastrin is an intestinal peptide that stimulates gastric parietal cells. The role of gastrin is in regulation of gastric acid secretion, and in controlling growth and differentiation of gastro-intestinal epithelial cells. There is a relationship between gastrin and the BO cell cycle, in which gastrin stimulates cell proliferation and the expression of COX-2 by activation of CCK2(17). With except of the induction of cell proliferation, invading apoptotic pathways in migration of canceric cells have also been reported(50). However, there is conflicting results on the effect of gastrin in the progression of BO to OA and still needs more investigation.

### Nitric Oxide

Studies showed that there is high level of nitric oxide (NO) in the oesophageal lumen as a consequence of denitrification of salivary nitrite by GORD acids. Acidic conditions convert nitrite to nitrous acid and nitrosating components, which are carcinogenic. The activity of NO has been reported in a number of biologic processes, in carcinogenesis, tumour progression, and DNA damage. The main effect of NO is in the S-phase of cell development,

which causes replication fork collapse due to DNA damage. In addition, high concentrations of NO can react with oxygen and produce N2O3, which alters DNA by nucleotide deamination or forming N-nitrosocompounds. It has been reported that NO and acid can cause double-strand breaks in oesophageal tissue, and BO, which may contribute to the progression of BO to OA(51,52). The role of NO in development of BO was studied in 1998, in which showed inducible NO synthase mediates inflammation and regulator of epithelial cell growth and its expression is high level in colorectal adenomas and carcinomas. Their findings from patient samples supported that the level of NO in patients with BO and OA is considerably higher than gastric control tissue(51,52).

### Inflammatory responses

Inflammatory responses have a close relationship with GORD, in the sequence of metaplasia- dysplasia-adenocarcinoma. The association between chronic inflammatory and carcinogenesis have been confirmed by many studies including Barrett's malignancy and gastrointestinal metaplasia, such as *Helicobacter pylori*-positive gastritis with atrophy intestinal metaplasia, intestinal and pancreatic metaplasia with carditis at the gastro-oesophageal junction, colonic metaplasia with ileal pouches, gastric metaplasia with duodenitis and coeliac disease, squamous metaplasia with gastric ulceration, ulcerative colitis with metaplastic polyps, and gastric carditis with intestinal metaplasia(5).

It is found that inflammatory mediators, cytokines and chemokines are released by injured oesophageal cells in response to damage to migrate inflammatory cells such as T lymphocytes, neutrophils, and NF-κB. NF-κB has an additional correlated role with inflammatory cells during BO, adenocarcinoma, and infrequently in oesophagitis(53). These indicate that there is a multiple pathway through the changes of BO to OA because, first, NF-κB can control regulation of pro-inflammatory mediator expression and growth regulatory cytokines (IL-8, 1β-4, and 1β-10) and TNF-α. In addition, it regulates various genes involving in cancer progression via apoptosis suppression, sustained proliferation and increasing migration, invasion, as well as angiogenesis. It has been reported that there are aberrant NF-κB activities in inflammatory disorders and cancer(54). TNF-α effects include enhancing the activity of the proto-oncogene c-myc pathway via β-catenin direction

at the metaplasia-dysplasia-adenoma sequence(43, 47,49,55). There is no difference between the amount of released interleukins in BO, but the inflammatory response changes from Th1 to Th2 and there are increased levels of IL-4 and IL- 6(43). Secondly, the influence of neutrophils in increasing BO malignancy is due the production of reactive oxygen species (ROS) that participate in DNA damage, such as in an ulcerative gastro-oesophageal mucosa. Finally, further activation of NF-κB by ROS and the TNF-α signalling pathway lead to increasing overall inflammatory responses and COX2 activity(56).

## Bacteria

It is assumed that more that 15% of carcinogenesis can be attributed to bacterial infection, such as *Helicobacter pylori* in gastric cancer and mucosal associated lymphoid tissue (MALT) lymphoma, *Salmonella typhimurium* in gallbladder cancer, *Streptococcus bovis* in colon cancer, and *Chlamydia pneumoniae* in lung cancer. Bacteria can cause chronic infections or produce toxins that help them to invade host cells, alter the cell cycle, mutate DNA, and control cell division and apoptosis, which can all lead to carcinogenesis. Damage to the immune system is another important way in which bacteria can induce mutagenic effects through the release of cytokines via inflammatory cells, for instance, IL-8, reactive oxygen species, COX2, and nitric oxide(57, 58). With respect to bacterial cell-cycle inhibitors (cytolethal distending toxins (CDTs)) and cell-cycle inhibitor factor (Cif) interfere with the immune system by blocking the clonal expansion of lymphocytes. Some bacteria induce cell proliferation and differentiation by cytotoxic necrotising factor (CNF). For example, CNF in *E.coli* activates $G_1$-S phase transition, and provokes DNA replication. Cif in enteropathogenic (EPEC) and enterohaemorrhagic *E.coli* (EHEC) arrests $G_2$-M phase that help bacterial attachment to the host cell. CDT in *Campylobacter jejuni* and *Salmonella typhi* block $G_2$ with unit of CDT, CdtB which is a DNase creating double-stranded DNA. In a PCR base study, 8 of 18 samples had significant counts of *S. anginosus*, which suggested that this organism might have a role in oesophageal and gastric cancer(57).

The effect of different bacterial groups in BO is unclear. Although the oesophageal epithelium is colonised by various types of bacteria, it is possible leading to damage of the epithelium by bacterial toxins, secretions, and other different mechanisms.

Few studies have investigated the role of bacteria in the aetiology of BO, but it has been reported that there is substantial overgrowth by Gram-positive and negative species. Other work on aspirated samples from BO patients and a control group showed that *campylobacters* were detected in large numbers, together with Gram-positive rods (*lactobacilli*, *bifidobacteria*, *propionibacteria*, *actinomyces*), Gram-positive cocci (*staphylococci*, *streptococci*, *gemella*, *rothia*), and other Gram-negative bacteria (*enterobacter*, *veillonella*, *neisseria*, *megasphaera*, *prevotella*, *fusobacteria*, *selenomonas*). Although *H. pylori* has been recognised as a duodenal ulcer and gastric cancer agent, it has not been detected in BO(20). In a similar study, 24 different bacterial species were detected on the oesophageal mucosa, with 14 bacterial homologous species, 5 unidentified homologs, and 5 unknowns. 17 of these organisms were found in GORD, 5 in BO, and 10 in normal oesophagus(59). Current findings indicate that with the exception of two groups of bacteria, Gram-positive and Gram negative, there is a shift from aerobic microbiota to Gram-negative anaerobic predominant microbiome at distal end of oesophagus. This study suggested that produced lipopolysaccharides by the second bacterial group, Gram negative, might induce GORD because of oesophageal sphincter relaxation through the nitric oxide synthesis, or gastric juice and microorganisms might cause distal oesophageal microflora changes(60). Evidence on oesophageal biofilm not only confirmed existing differences on oesophageal and BO biofilm on Macfarlane's study(20), it also revealed the role *C.concisus* as a novel pathogenic bacterium. Their findings specify effect of *C.concisus* on elevation of cytokines expression(61). Current study on the putative role of C. concisus in the expression of biomarkers involved on the progression of BO transition such as IL-18, TNFα, p53, CDX1, and COX2 demonstrated that organism could modulate expression of molecular markers on cell culture model of Barrett's cell lines(62-63).

In conclusion, using current technical procedures have made possible to study molecular and cellular markers and mechanism that might involve in the progression of Barrett's disease. Although studies have considered different factors that may contribute in Barrett's transformation, there is no clarified factor that functionally clarifies molecular and cellular changes of Barrett's epithelium to the adenocarcinoma. Review provides collection of data to show weather selected investigating markers could

have established progression of BO to the oesophageal adenocarcinoma or identifying new factors might gain new insights into the pathogenesis of Barrett's disease. However, it is obvious that further research in this field is required to study about the role of bacteria involved in Barrett's epithelium.

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