Molecular Biology of Barrett's Esophagus:
From Bench to (Almost) Bedside

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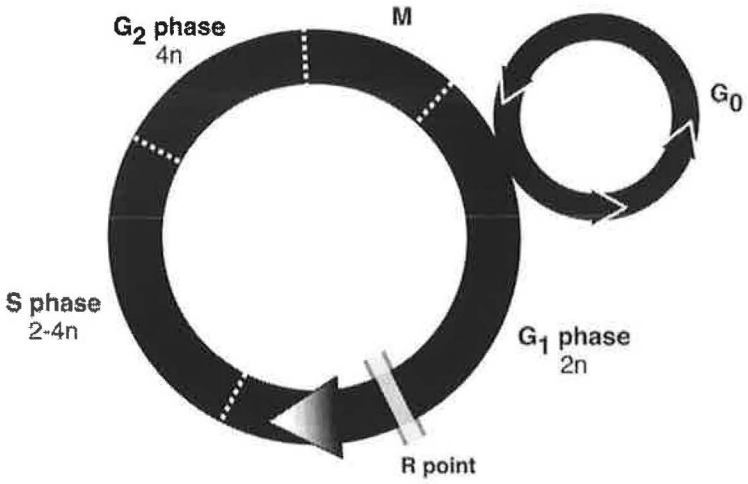
At the Bench:

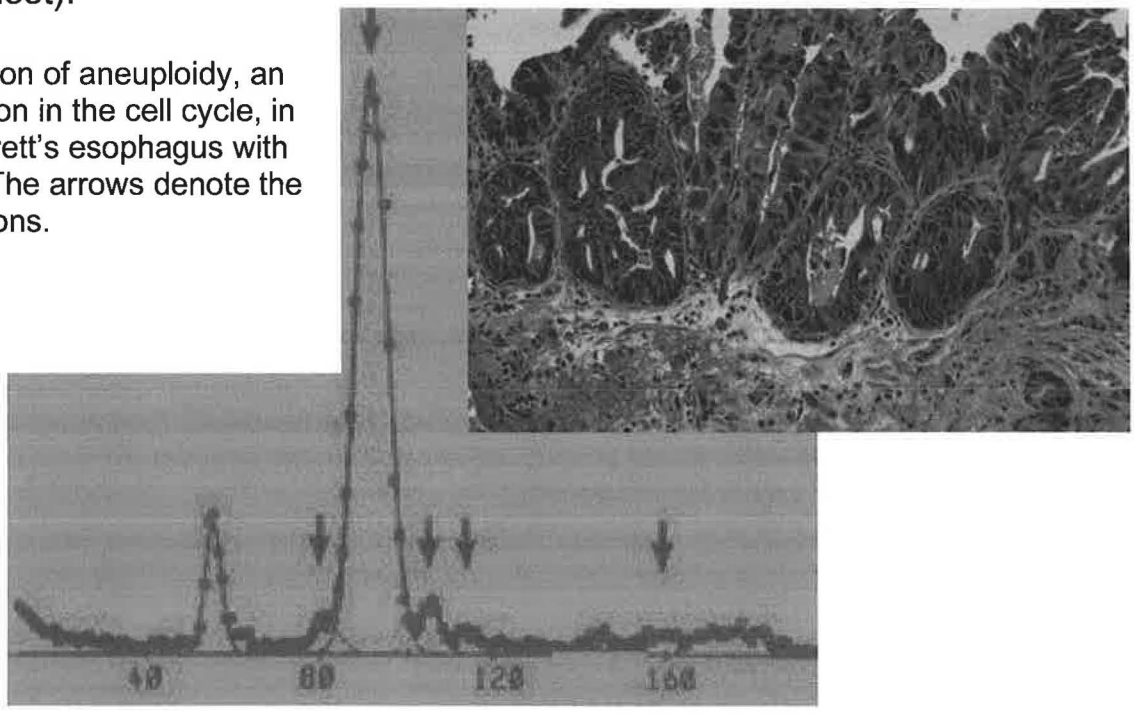
Understanding the Cell Cycle



At the Bedside (Almost):

Flow cytometric detection of aneuploidy, an indication of an alteration in the cell cycle, in a tissue sample of Barrett's esophagus with high grade dysplasia. The arrows denote the aneuploid cell populations.

High Grade Dysplasia in Barrett's Esophagus



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My major interests are in the molecular mechanisms underlying the development and neoplastic progression of Barrett's esophagus. In our laboratory, one group of studies utilizes biopsy tissues and cell culture systems to delineate the signal transduction pathways activated by acid, bile, and the combination of both in esophageal squamous and metaplastic Barrett's epithelia. In addition, the downstream effects on proliferation and apoptosis of these activated signal transduction pathways are investigated in an effort to identify potential targets at which to direct chemopreventive and chemotherapeutic agents. Our laboratory is also using microarray technology to identify novel genetic alterations that may predispose patients to the development of Barrett's esophagus in the setting of chronic gastroesophageal reflux. In collaboration with Dr. Ruben Ramirez, Assistant Professor of Medicine, we are developing novel tissue equivalents of esophageal squamous and metaplastic Barrett's mucosa in order to assess the effects of exposure to acid, bile, or both on the stratification and differentiation of these esophageal epithelial cells. Lastly, in collaboration with Dr. George Sarosi, Assistant Professor of Surgery, we are developing a rat model of Barrett's esophagus and esophageal adenocarcinoma in order to test *in vivo* potential chemopreventive and chemotherapeutic agents, identified by our *in vitro* studies, in the development and neoplastic progression of Barrett's esophagus.

This is to acknowledge that Dr. Souza has disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr Souza will not be discussing off-label uses in her presentation.

Introduction

Esophageal cancer is one of the most deadly forms of gastrointestinal cancer with a mortality rate exceeding 90%. The major risk factors for esophageal adenocarcinoma are gastroesophageal reflux disease (GERD) and its sequela, Barrett's esophagus. (1) GERD most commonly leads to esophagitis, in a minority of patients however, ongoing GERD leads to replacement of esophageal squamous mucosa with metaplastic, intestinal-type Barrett's mucosa. In the setting of continued peptic injury, Barrett's mucosa can give rise to esophageal adenocarcinoma. (1; 2) Despite the widespread use of potent acid suppressive therapies for patients with GERD, the incidence of esophageal adenocarcinoma, among white men in the United States has continued to rise over the past three decades.(3)

Endoscopic surveillance of patients with known Barrett's esophagus is the current strategy to manage the risk of cancer formation in this at risk patient group. However, a recent study suggested that the majority of patients diagnosed with esophageal adenocarcinoma were unaware that they had Barrett's esophagus and thus, we not enrolled in surveillance programs. (4) Therefore, our current management strategy for the early detection of cancer in patients with Barrett's esophagus appears to be ineffective. Another problem with our current strategy is that it is targeted at the detection of dysplasia in the Barrett's mucosa. However, dysplasia is an imperfect predictor of cancer risk in Barrett's esophagus. Some of the current problems in using dysplasia as an indicator of cancer risk include the poor intra- and inter-observer reproducibility of dysplasia interpretations and the poor predictive value for negative, indefinite, low grade, and even high grade dysplasia. (5-7) Dysplasia is a conglomerate of histologic abnormalities suggesting that cells have acquired DNA damage rendering them neoplastic and predisposed to cancer formation. Therefore, dysplasia is essentially the histologic expression of DNA damage that preceeds malignancy thereby making the detection of dysplasia an intermediate or even late indicator of neoplastic progression rather than a truly early indicator of cancer risk. Therefore, a better indicator of cancer risk would be detection of the genetic damage itself before the histologic manifestations of dysplasia are even apparent.

With the recent advances in molecular biology, efforts to characterize the specific molecular events which occur during the evolution of esophageal adenocarcinoma have intensified. The identification of molecular biomarkers may offer easy reproducibility and standardization in addition to the truly early detection of neoplastic progression. Therefore, it has become increasingly important to understand the pathogenesis of Barrett's esophagus and esophageal adenocarcinoma at the molecular level in order to identify target biomarkers for the diagnosis of this deadly disease. At the molecular level, the evolution of esophageal adenocarcinoma from metaplastic Barrett's esophagus requires a sequence of genetic alterations in which normal esophageal cells acquire the six physiologic hallmarks of cancer progression proposed by Hanahan and Weinberg in 2000. (8) These cancer hallmarks include the ability to proliferate without exogenous stimulation, to resist growth-inhibitory signals, to avoid triggering the programmed death mechanism (apoptosis), to resist cell senescence, to develop new vascular supplies (angiogenesis), and to invade and metastasize. These hallmarks represent the physiologic traits that must be acquired by cells during the genesis of all human tumors. Therefore, these hallmarks are not specific for neoplastic progression of Barrett's esophagus. However, as each of these hallmarks is reviewed, particular attention will be paid to the genetic alterations that occur in Barrett's cells that contribute to the acquisition of each of the hallmarks.

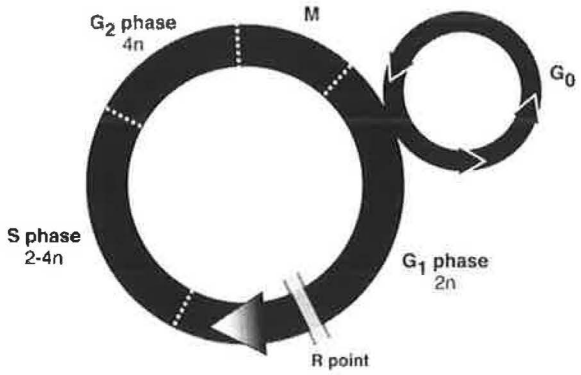
The Cell Cycle

Acquisition by Barrett's cells of the cancer hallmarks which encompass the ability to proliferate without exogenous stimulation, to resist growth-inhibitory signals, and to avoid triggering apoptosis involve events which allow the cell to overcome the normal restrictions placed on cell growth. Therefore, before delineating abnormalities acquired by cells to overcome the restrictions placed on normal growth, a brief overview of normal cell growth and its regulatory points is in order.

The cell cycle is the pivotal molecular machinery in the cell nucleus that controls whether a cell will proliferate, differentiate, become quiescent or die. The cell cycle comprises the intranuclear events that occur during the period between two mitotic divisions (Figure 1). The cycle is divided into 4 phases called G1 (first gap), S (DNA synthesis), G2 (second gap), and M (mitosis). The RNA and proteins needed for DNA replication are synthesized during the G1 phase. There is a critical point, late in the G1 phase, where a decision is made either to continue and complete the cell cycle, or to exit the cycle. This critical juncture is called the 'restriction point' (R-point). (9) In S phase, DNA replication takes place and the cell's DNA content doubles from the diploid value of 2n to the fully replicated, tetraploid value of 4n. The tetraploid cell prepares for the upcoming mitotic division in G2 phase. Finally, in M phase the cell divides into two daughter cells, each containing a diploid (2n) complement of DNA. Cells may also withdraw from the cell cycle to enter a quiescent state termed G0. Under certain conditions, such cells can be stimulated to leave the G0 phase and reenter the cell cycle.

Cellular proliferation has been studied in biopsy specimens of Barrett's esophagus using a variety of markers including tritiated thymidine incorporation, and immunostaining for Ki-67 and PCNA.(10; 11) Barrett's esophagus has been found to have an increase in these markers compared to gastric fundic and junctional-type epithelia suggesting that Barrett's esophagus has an increased proportion of cells entering into the cell cycle and hence undergoing proliferation. While there are a number of regulatory points throughout the cell cycle, the two primary points are at the transition from G2 to M phase and from G1 to S phase. It is the transition from G1 to S phase, requiring passage through the R point, that appears to be the most highly regulated and the most well studied. Although many proteins have been implicated in the regulation of this critical transition point, the retinoblastoma (Rb) protein appears to be the molecular switch that regulates passage through the R-point.

Figure 1. Overview of the cell cycle.
G0, G1, R point, S, G2, and M refer to the quiescent state, first gap, restriction point, DNA synthesis, second gap and mitosis phases of the cell cycle respectively. *n* refers to the chromosome number. (*From* Souza, RF. A conceptual approach to understanding the molecular mechanisms of cancer development in Barrett's oesophagus. Alimentary Pharmacology and Therapeutics 2001; 15: 1087-1100; with permission.)



Retinoblastoma protein: Master Switch in Control of the R-Point

Normally, Rb protein is in the active, hypophosphorylated form which blocks R-point progression (Figure 2). (12) Following phosphorylation, Rb becomes inactive, thereby allowing the cell to pass through the R-point into the remainder of the cell cycle. Although mutation of the Rb gene in Barrett's esophagus or in Barrett's-associated adenocarcinomas has not yet been demonstrated, multiple studies suggest that alterations in Rb function play a role in neoplastic progression of Barrett's esophagus. (13-15) Now with some background on cell cycle regulation, lets turn our attention to how Barrett's cells may acquire each of the 6 physiologic hallmarks of cancer formation.

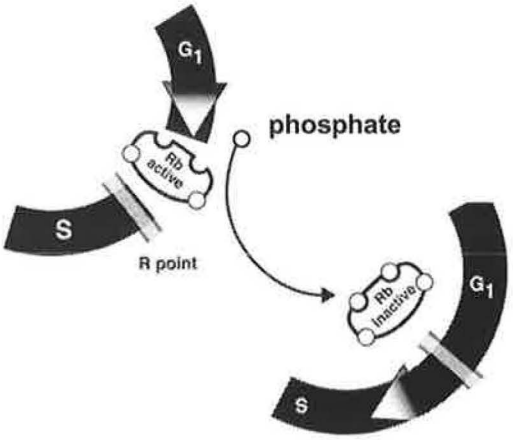


Figure 2. Retinoblastoma (Rb) is the molecular switch in control of the R point. Hypophosphorylated Rb blocks progression through the R point. Following phosphorylation, Rb is inactivated and cell cycle progression can proceed.

Six Physiologic Hallmarks of Cancer Progression in Barrett's Esophagus

Cancer Hallmark #1. The ability of Barrett's cancer cells to proliferate without exogenous stimulation.

One mechanism whereby cells can proliferate without exogenous stimulation is by the expression of oncogenes. Proto-oncogenes are normal cellular genes that stimulate cell growth. When these proto-oncogenes become mutated in such a way that they become overactive, they are called oncogenes. Two examples of oncogenes implicated in Barrett's esophagus are cyclins D1 and E. Phosphorylation of Rb occurs by interactions with cyclin-dependent kinases (cdks) and cyclins (Figure 3). (12) Cdks are the enzymes responsible for the phosphorylation of Rb whereas cyclins act as shuttle proteins to carry the cdks to their protein targets. Cdks and cyclins ultimately regulate the transitions from G1 to S phase as well as from G2 to M phase. Cyclins D1 and E, which mediate the G1 to S phase transition, and cyclin B1, which acts at the G2 to M transition, have been implicated in neoplastic progression in Barrett's esophagus. (16-19)

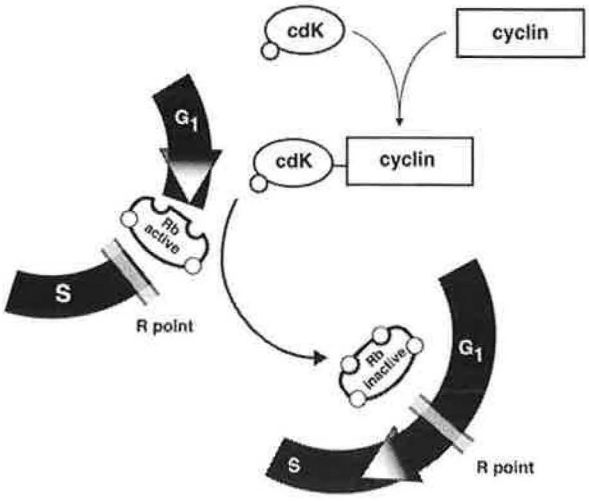


Figure 3. Cdks and cyclins regulate Rb phosphorylation. Cyclins form complexes with cdks which phosphorylated and inactive Rb. Inactivation of Rb then allows the cell cycle to proceed.

Increased nuclear expression of cyclin D1 protein has been detected in biopsy specimens of non-dysplastic Barrett's metaplasia compared to normal squamous controls. (17) In contrast, overexpression of cyclin E has been found in both low grade and high grade dysplastic areas of Barrett's esophagus as well as in adenocarcinomas in Barrett's esophagus, but not in non-dysplastic Barrett's samples. (18) Expression of cyclin B1 has been detected in non-dysplastic and dysplastic samples of Barrett's esophagus as well as in esophageal adenocarcinomas. (19) Unfortunately, the expression of cyclin B1 in control tissues such as normal esophageal or intestinal-type tissue was not examined, making the role of cyclin B1 expression in the neoplastic progression of Barrett's esophagus questionable. (19)

Another mechanism whereby Barrett's cells can proliferate without exogenous stimulation is by altering growth factors, growth factor receptors, or the signal transduction pathways activated in response to growth factor-receptor interactions. The binding of growth factors to receptors that are members of the tyrosine kinase family can promote cellular proliferation by activating signal transduction cascades initiated by the activation of Ras which eventually converge on cyclin D1 (Figure 4). (20; 21) The Ras/Raf/mitogen activated protein kinase (MAPK) pathway is one of the key growth-stimulating signaling cascades activated when growth factors bind their tyrosine kinase receptors (Figure 4). (22) The induction of cyclin D1, as a result of these growth-stimulating signaling cascades, ultimately facilitates cell cycle progression.

Increased expression of epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), have been found in metaplastic Barrett's esophagus and have been implicated in the development of adenocarcinomas in Barrett's esophagus. (23-25) Increased expression of the EGF receptor (also called ErbB-1) has been detected in specialized intestinal metaplasia and in esophageal adenocarcinomas. (23-25) Controversy exists regarding the role of an oncogenic form of the normal receptor tyrosine kinase erbB-2 termed *erb*B-2 (also called HER2 or Neu) in the development of Barrett's-associated adenocarcinomas. (26-28) In a number of extra-esophageal tumors, ras proteins (including H-ras and K-ras,) have been identified as important human oncogenes. (29)However, available data do not support an important role for oncogenic ras in Barrett's-associated cancers.(30; 31)

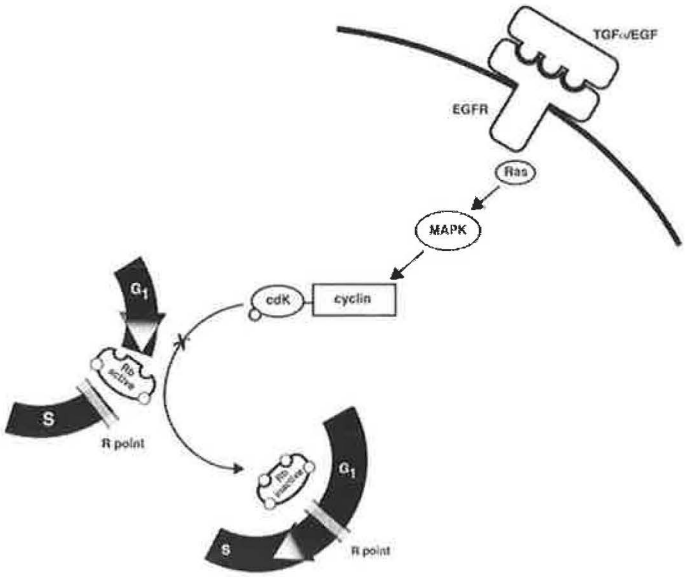


Figure 4. Binding of growth factors TGF-α and EGF with EGFR promotes cell cycle progression by activation of ras and the MAPK pathways. Activation of the MAPK pathways lead to induction of cyclin D1 which phosphorylates Rb thereby facilitating cell cycle progression. (*From* Souza, RF. A conceptual approach to understanding the molecular mechanisms of cancer development in Barrett's oesophagus. Alimentary Pharmacology and Therapeutics 2001; 15: 1087-1100; with permission.)

Cancer Hallmark #2. The ability of Barrett's cancer cells to resist growth-inhibitory signals.

Antigrowth signals can block cell proliferation by preventing passage through the R point. Most anti-growth signals converge on the Rb pathway to prevent phosphorylation and subsequent inactivation of Rb. Tumor suppressor genes are normal genes that restrain the cells ability to proliferate by preventing phosphorylation of Rb. When tumor suppressor genes are inactivated, the pathways leading to Rb phosphorylation become un-opposed, allowing for uncontrolled proliferation. Tumor cells can inactivate tumor suppressor genes by at least three mechanisms, including mutation, deletion of the chromosomal region containing the gene (called loss of heterozygosity (LOH)), or by attachment of methyl groups to the promoter region of genes (called promoter methylation). No studies yet have demonstrated mutation of the Rb gene in Barrett's esophagus or in Barrett's-associated adenocarcinomas. However, loss of allele 13q, the locus for the Rb gene, altered Rb messenger RNA (mRNA) transcript size and quantity, and loss of immunostaining for Rb have been demonstrated in both Barrett's-associated dysplasia and adenocarcinomas. (13-15) Although not conclusive, these data suggest that altered Rb function plays a role in neoplastic progression of Barrett's esophagus.

The tumor suppressor genes p53, p16, and p15 have also been implicated in the development of Barrett's-associated adenocarcinomas. When the DNA of normal cells sustains damage during G1 phase of the cell cycle, p53 protein rapidly accumulates to halt further progression of the cell cycle by inhibiting Rb phosphorylation by cyclin D1 (Figure 5). (32) p16 and p15, members of the INK4 family of tumor suppressor genes, also act to block phosphorylation of Rb by cyclin D1 (Figure 5). (33; 34) The cell cycle arrest induced by p53, p16, and p15 allows time for the cell to repair the damaged DNA before the mutation can be propagated by DNA replication during S phase. If a cell loses its normal function of any of these tumor suppressor genes, then DNA damage might not be repaired, and the mutation can be perpetuated thereby facilitating carcinogenesis.

The role of p53 in the neoplastic progression of Barrett's esophagus has been widely studied. Inactivation of p53 by LOH of 17p, the p53 locus, and mutation of the remaining allele has been found in approximately 50-90% of esophageal adenocarcinomas in Barrett's esophagus. (35-37) LOH of 17p also has been detected in the nonmalignant, diploid cells of specialized

intestinal metaplasia, suggesting that inactivation of p53 is an early step in carcinogenesis in Barrett's esophagus. (38)

Barrett's-associated adenocarcinomas frequently demonstrate allelic loss of 9p21, the chromosomal locus for p16 and p15. (39) In addition, LOH of 9p21 has been found in 90% of premalignant (aneuploid) Barrett's epithelium. (40) However, the mere finding of LOH at 9p21 does not establish that p16 and p15 are the specific targets of the deletion. The demonstration of point mutations in the remaining allele traditionally has been taken as evidence that the altered gene is the target of deletion. No point mutations for p15 have been found in Barrett's esophagus, suggesting that this gene may not play a major role in esophageal carcinogenesis. (41) In esophageal adenocarcinomas, homozygous deletion of the p16 gene and point mutation of the remaining allele of p16 has been described but only in a few cases. (39; 41) An alternative mechanism for silencing a tumor suppressor gene (other than LOH and mutation) is that of promoter methylation. Methylation of the p16 promoter has been found in 45% of esophageal adenocarcinomas. (40)Moreover, methylation of p16 has been detected in non-dysplastic, specialized intestinal metaplasia suggesting that p16 methylation is the earliest event in the neoplastic progression of Barrett's esophagus. (42)

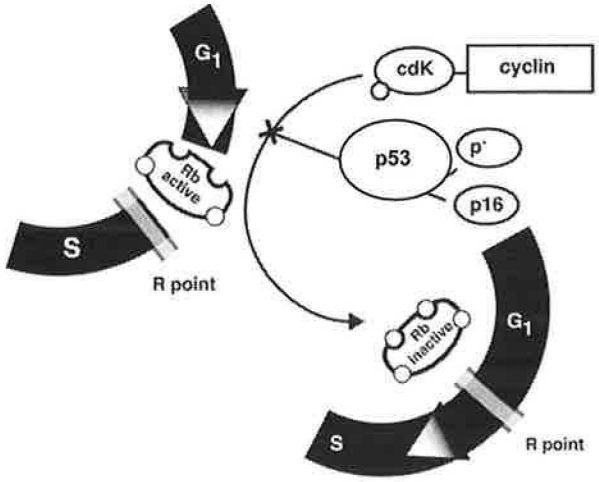


Figure 5. p53, p16, and p15 (not shown) inhibit cell cycle progression by preventing Rb phosphorylation by the cyclins and cdks. By inhibiting Rb phosphorylation, p53, p16, and p15 induce G1 arrest and prevent passage through the R point.

Another tumor suppressor gene implicated in the neoplastic progression of Barrett's esophagus is the adenomatous polyposis coli gene (APC). APC contains a binding site for β-catenin, a protein involved in cellular signal transduction. In response to activation of the β-catenin signal transduction pathway, β-catenin accumulates in the cytoplasm and moves to the nucleus where it binds and activates the T cell factor/lymphoid enhanced binding factor-1 (TCF/LEF-1) family of transcription factors that mediate the expression of growth related genes (Figure 6). (43) In the absence of activation of the β-catenin signal transduction pathway, β-catenin becomes rapidly degraded in a process that involves the APC protein. (44) Therefore, tumorigenesis might result from inhibition of the interaction between β-catenin and APC leading to an increase in β-catenin signaling.

Esophageal adenocarcinomas commonly demonstrate LOH of 5q21, the APC locus, but only rare mutation in APC. (45) APC promoter methylation is more common with 83-92% of Barrett's high grade dysplasia and esophageal adenocarcinomas and 40-50% of Barrett's metaplasias without dysplasia demonstrating APC inactivation by this mechanism. (46) In addition, methylated APC DNA has been found in the plasma in 25% of patients with esophageal

adenocarcinoma, and has been associated with a significantly shortened patient survival. (47)

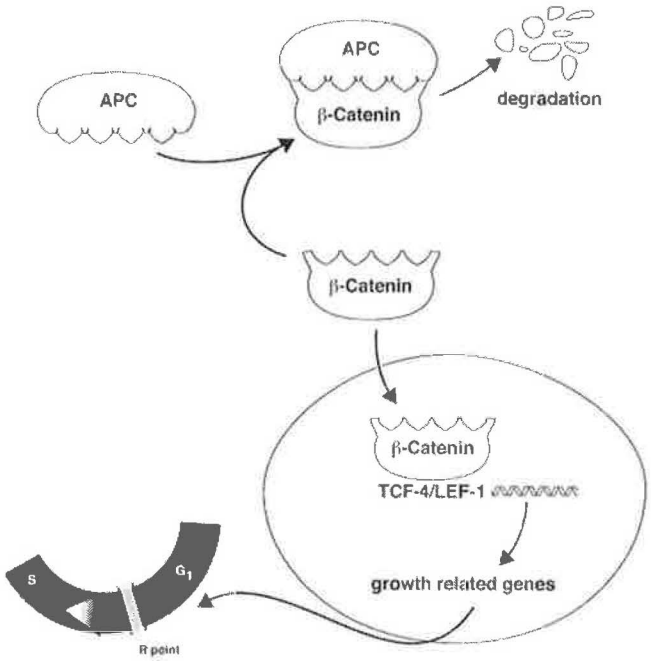


Figure 6. APC and β-catenin signaling. Mitogenic signals facilitate the movement of β-catenin into the nucleus where it activates the TCF-4/LEF-1 family of transcription factors to produce growth related gene products. In the absence of mitogenic signals, β-catenin binds to APC and is targeted for degradation. (*From* Souza, RF. A conceptual approach to understanding the molecular mechanisms of cancer development in Barrett's oesophagus. Alimentary Pharmacology and Therapeutics 2001; 15: 1087-1100; with permission.)

Cancer Hallmark #3. The ability of Barrett's cancer cells to avoid triggering the programmed death mechanism (apoptosis).

Apoptosis is an innate, cellular self-destruct mechanism encoded in all normal cells. Normally, apoptosis is beneficial in that it prevents cells with damaged, mutated DNA from undergoing replication. However, to cancer cells, apoptosis is detrimental and cell cells must find ways to overcome this suicide program. The apoptotic machinery comprises several death-commitment signaling pathways which can be activated by DNA damage, metabolic abnormalities, and death receptor activation. Irregardless of the mechanism of activation, the death-commitment pathways converge on a common executioner pathway that ultimately destroys the cell through a caspase signaling cascade. (48)

Barrett's-associated adenocarcinomas have found ways in which to overcome triggering apoptosis by disruption of a variety of the death-commitment pathways. As already discussed, inactivation of p53 is one way in which Barrett's cancer cells avoid inducing apoptosis as a result of DNA damage or mutation. The expression of 13-S-hydroxyoctadecadienoic acid (13-S-HODE), a fatty acid that is formed from linoleic acid through the action of 15-lipoxygenase-1 (15-LOX-1), normally activates the apoptotic machinery. Decreased expression of 15-LOX-1, a potential means for avoiding apoptosis, has been found in 75% of Barrett's-associated esophageal adenocarcinomas. (49)

Death receptors are another activator of the death-commitment signaling pathways. The binding of the cell surface death receptor, Fas, with Fas-ligand (FasL), a death-promoting ligand, activates the apoptotic cascade. (50) Normally, the Fas death receptor is found on the surface of both lymphocytes and gut epithelial cells, whereas FasL is expressed by activated lymphocytes but not by epithelial cells. When FasL binds to the Fas receptor, apoptosis is induced in the cell expressing the Fas receptor. FasL expression using immunohistochemistry has been found in one

study of 13 esophageal adenocarcinomas. (51) By expressing FasL, these tumor cells are now capable of binding the Fas receptor on the surface of attacking lymphocytes, thereby destroying the tumor killing immune cells.

Finally, another mechanism whereby Barrett's cancer cells might avoid apoptosis is by increasing the synthesis of an agent that normally blocks the death-commitment signaling pathways such as cyclooxygenase-2 (COX-2). Overexpression of COX-2 reduces the rate of apoptosis *in vitro*,(52; 53) and COX-2 overexpression has been detected both in esophageal adenocarcinomas and in the metaplastic epithelium of Barrett's esophagus. (53; 54)

Cancer Hallmark #4: The ability of Barrett's cancer cells to resist cell senescence.

To become immortal, tumor cells must overcome intrinsic mechanisms that limit the proliferative capacity of normal cells. This autonomous, intrinsic mechanism for cell senescence involves shortening of telomeres. Telomeres are long stretches of simple, non-coding DNA repeats located on the ends of chromosomes. During each successive round of cell replication, telomeric DNA is lost. Eventually, short telomeres trigger an exit from the cell cycle at G1 and entry into senescence, a G0 state characterized by permanent growth arrest. Therefore, in order for cells to become immortal, they must synthesize new telomeres. Telomerase is the enzyme responsible for the synthesis of new telomeres. (55) Telomerase is a protein-RNA complex that uses its RNA as a template for the addition of telomeric sequences to the ends of chromosomes. Most normal esophageal cells and tissues lack telomerase, however, Barrett's-associated adenocarcinomas demonstrate high levels of telomerase expression. (56) In contrast, benign Barrett's esophagus expresses low levels of telomerase which appears to increase as the metaplastic cells progress to high grade dysplasia. (57)

Cancer Hallmark #5: The ability of Barrett's cells to develop new vascular supplies (angiogenesis).

The formation of new blood vessels (angiogenesis) is essential for further growth and metastasis of tumors. The vascular endothelial growth factors (VEGFs) are a family of potent angiogenic factors. Upon binding to their receptors, the vascular endothelial growth factor receptors (VEGFRs), VEGFs initiate signal transduction pathways that result in the proliferation and migration of endothelial cells. The expression of VEGF-A has been found in the epithelial cells of metaplastic Barrett's esophagus, but not in normal squamous esophagus. Expression of corresponding receptor for VEGF-A, the VEGFR-2, has also been found in the blood vessels feeding metaplastic Barrett's epithelium. (58) In addition, VEGF-C expression has been found in metaplastic Barrett's epithelium, while neoplastic Barrett's tissues demonstrate expression of VEGFR-3. (59)

Cancer Hallmark #6. The ability of Barrett's cancer cells to invade and metastasize.

Although the mechanisms whereby cancer cells invade and metastasize are poorly understood, abnormalities in cell-cell interaction are thought to play a role. Cadherins, a large family of cell adhesion molecules, bind to cytoplasmic proteins called catenins that are linked to the cell's actin cytoskeleton. (60) Processes that prevent the interaction of cadherins and catenins can impair cell adhesion and predispose to invasion and metastasis. In addition to its role in signal transduction, β-catenin is also involved in cell-cell interactions in the esophagus. In normal esophageal squamous mucosa and the non-dysplastic, specialized intestinal metaplasia of Barrett's esophagus, E-cadherin and β-catenin are found primarily in the cell membrane. (61; 62)

Studies of dysplastic Barrett's esophagus have shown a decrease in E-cadherin and β-catenin in the membrane, with an increase in cytoplasmic and nuclear staining for these proteins.(63) Furthermore, memberane staining for E-cadherin and β-catenin appears to fall as the degree of dysplasia increases. (64)

From Bench To (Almost) Bedside: Clinical Implementation of Barrett's Molecular Biology

Although a large number of biomarkers have been identified in Barrett's esophagus only a few of these will prove eventually to be clinically useful. The National Cancer Institute's Early Detection Network (EDRN) has proposed 5 phases of study that biomarkers must undergo for validation. (65) It is only in the latter 3 phases, that clinical studies are carried out to (1) evaluate retrospectively the ability of the biomarker to detect preclinical disease and define criteria for a "positive" test (phase 3), (2) determine the ability of the biomarker to predict disease outcomes prospectively (phase 4), and (3) estimate the reduction in mortality by action taken based on the biomarker assay (phase 5). (66) No Barrett's biomarkers have been evaluated in phase 5 studies. The only Barrett's biomarkers to have been examined in phase 4 studies include cell cycle abnormalities detected by flow cytometry (aneuploidy and tetraploidy) and p53 LOH. While the results of the phase 4 studies are promising, the use of these biomarkers in routine clinical practice is not yet recommended. (66)

Aneuploidy refers to an alteration in DNA content other than the normal diploid (2n) or tetraploid (4n). Aneuploidy indicates genomic instability and an increased risk of neoplastic progression. Aneuploidy can be detected by flow cytometry. While tetraploid cell populations can be considered normal, tissues containing more than 6% of the total cell population as tetraploid cells are considered at an increased risk to progress to aneuploidy. Tissues containing more than 6% of the total cells in the tetraploid state are also referred to as containing an elevated 4n fraction. (67; 68) Flow cytometric abnormalities and histology as predictors of progression to adenocarcinoma have been evaluated in a large phase 4 study. (68) Barrett's esophagus patients had histology and flow cytometry performed on biopsies obtained upon entry into the study (baseline). The patients were then followed for 5 years and the incidence of cancer formation was assessed. In patients with aneuploidy (populations with over 2.7N) at baseline, the 5 year incidence of cancer progression was 64%; in patients with elevated 4n fractions (tetraploidy), the 5 year cancer incidence was 57%; and in patients with both aneuploidy and tetraploidy, it was 75%. (7; 68)On the other hand, the rate of cancer incidence in patients without aneuploidy or tetraploidy was only 5.2% and all of these patients had high grade dysplasia. Flow cytometry was most useful in predicting cancer progression in patients with no dysplasia, indefinite, or low grade dysplasia in the biopsy specimens obtained at baseline. The detection of either aneuploidy or tetraploidy heralded a 5 year cancer incidence of 39% compared to 0% in patients with neither of these flow cytometric abnormalities. (68)

p53 LOH is the other Barrett's biomarker to be evaluated in a large phase 4 study. (69) Patients with Barrett's esophagus had histology and LOH of p53 determined on biopsies obtained at baseline, upon entry into the study. Patients were then followed for 5 years and the incidence of cancer formation was assessed. p53 LOH was a significant predictor of cancer progression at 5 years ($p<0.001$, RR 16), however this patient group included those with high grade dysplasia.(69) In patients with no dysplasia, indefinite, or low grade dysplasia, p53 LOH remained a significant predictor of progression to high grade dysplasia or cancer ($p=0.02$).(69)

Summary

This protocol describes how the genetic abnormalities that have been recognized in Barrett's esophagus might allow the cells to acquire the 6 physiologic hallmarks of cancer described by Hanrahan and Weinberg in 2000 (Figure 7). This approach provides a useful conceptual basis for evaluating studies on the molecular mechanisms underlying the progression from metaplasia to carcinoma, and for developing cancer treatment and preventive strategies that are based on the molecular biology of the tumor. However, that the genetic alterations described in this protocol represent only a fraction of the changes required for a benign cell to acquire these cancer hallmarks. Furthermore, the interactions among oncogenes, tumor suppressor genes, growth factors, and signal-transduction cascades undoubtedly are far more complex than are currently presented. The elucidation of the basic mechanisms underlying carcinogenesis in Barrett's esophagus has lead to the identification of potentially useful clinical biomarkers. Although the routine clinical use of biomarkers is not yet recommended, it is anticipated that in the next few years there will be an increase in the number of validated biomarkers and that movement into the clinics seems inevitable. All together, it seems reasonable to assume that elucidation of the basic mechanisms of carcinogenesis will lead to clinical advances and improved outcomes for patients with adenocarcinoma in Barrett's esophagus.

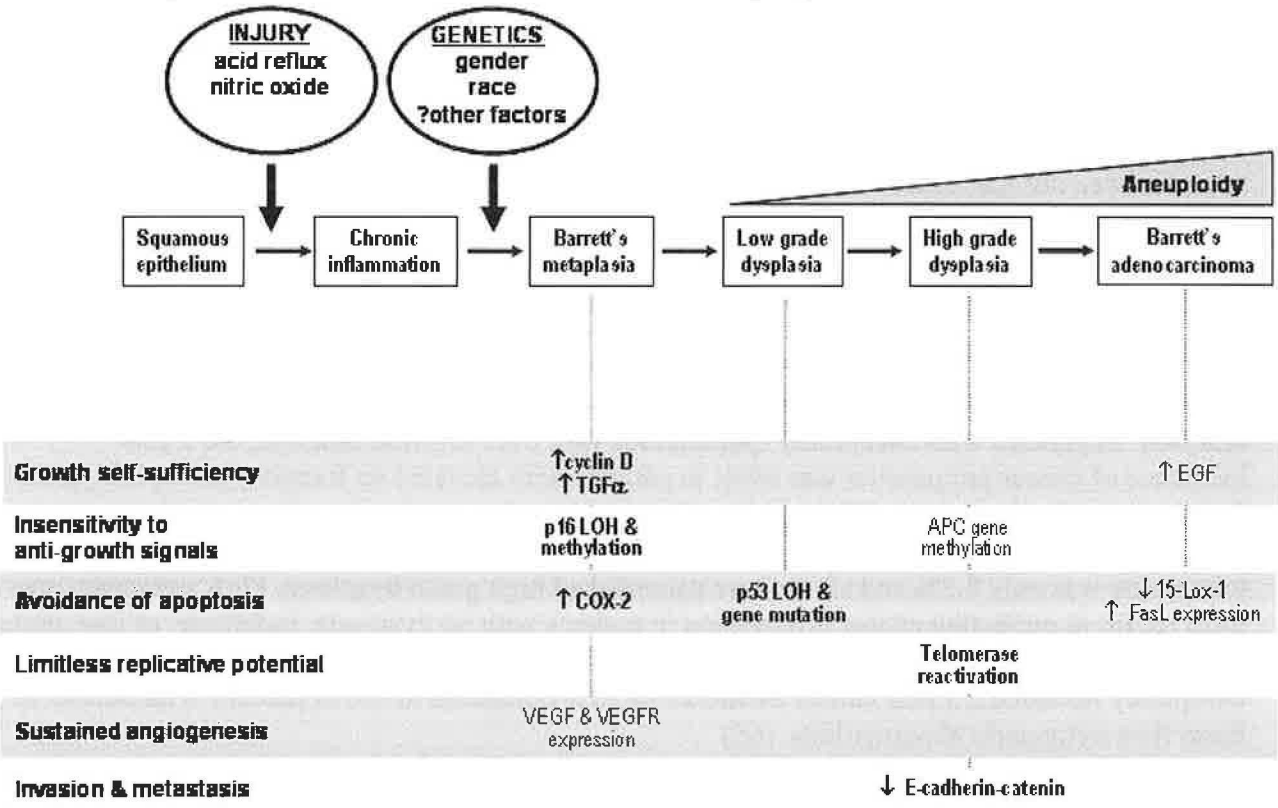


Figure 7. Major genetic alterations acquired by Barrett's cells during neoplastic progression to esophageal adenocarcinoma. The histologic stage at which each genetic change has been recognized is depicted. The alterations in bold type indicate those that have the strongest link to carcinogenesis. The contribution of host and environmental factors in the initiation of metaplastic Barrett's esophagus is also shown. (From Morales CP. Hallmarks of cancer progression in Barrett's oesophagus. Lancet 2002; 360: 1587-1589; with permission.)

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