

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Gastroesophageal Reflux Disease: Molecular Predictors in Neoplastic Progression of Barrett's Esophagus

Fritz Francois, Abraham Khan,  
Liying Yang, Sam M. Serouya and Zhiheng Pei  
*New York University Langone Medical Center*  
USA

## 1. Introduction

Barrett's esophagus (BE) represents a metaplastic change from squamous epithelium to intestinal epithelium as a result of chronic gastroesophageal reflux. Since the development of esophageal adenocarcinoma (EAC) is not universal among patients with BE, it is important to understand and to gauge the factors that influence risk of progression to dysplasia and cancer. While heartburn symptoms have been reported to be associated with BE (Eisen et al., 1997; Lagergren et al., 1999a), the severity of gastroesophageal reflux symptoms is not a reliable indicator for the presence of BE (Eloubeidi and Provenzale, 2001). There is a vital need to explore factors other than symptoms that not only may elucidate the pathophysiology of BE development but also that may be predictive of progression to EAC. Significant advances have been made along key areas such as cell cycle abnormalities, growth factors, adiposity, and the gut microbiome. This chapter aims to review some of these elements as well as the prognostic value of biomarkers for progression from BE to EAC. The importance of fulfilling the promise that these biomarkers hold is underscored by the notable increase in the risk of progression to cancer from 0.5% per year in non-dysplastic BE, to 13% in the setting of low-grade dysplasia, and to 40% in high-grade dysplasia (Curvers et al., 2010; Wani et al., 2009).

## 2. Cell cycle abnormalities

The normal cell cycle by which cells proliferate is comprised of an intricate system of checkpoints and regulations designed to carefully modulate growth. Cell cycle regulation is dependent on the members of several protein classes, including cyclins and cyclin dependent kinase (CDK) complexes, tumor suppressors, and pro as well as anti-apoptotic proteins. Derangements in this system result in dysregulation of the cell cycle and provide opportunity for uncontrolled proliferation, as well as the potential for neoplastic progression (Evan and Vousden, 2001). The tissue invasion and metastatic progression phases of the neoplastic change are also dependent on cellular as well as extracellular proteins that are normally involved in cell cycle regulation. The pathophysiologic mechanisms through which these proteins function have been implicated in most cancers

and are the focus of current research due to their possible prognostic value and implication for therapeutic targeting. The association of chronic GERD with the development of BE, dysplasia, and EAC (Gerdes, 1990; Herbst et al., 1978; Pellish et al., 1980; Preston-Martin et al., 1990; Reid et al., 1993; Ronkainen et al., 2005) provides an opportunity to explore the underlying cellular mechanisms that drive the transformation. This section will discuss the mechanisms by which cell cycle proteins may confer a survival advantage for transformation of BE to EAC.

As a class, tumor suppressor genes code for proteins that protect the cell by arresting the cellular growth cycle or by promoting apoptosis. Proteins such as p53, p16, and adenomatous polyposis coli (APC), normally recognize DNA damage and halt progression through the cell cycle, allowing for repair, senescence, or cell death. Therefore allelic mutations leading to loss of function of these proteins can be carcinogenic (Sherr, 2004). Mutations of the p53 protein have been implicated in nearly every cancer and may be one of the most common derangements in BE and EAC (Greenblatt et al., 1994; Vaninetti et al., 2008). Normally the detection of DNA damage results in p53 activation via signals that promote p21 transcription with subsequent binding and inhibition of cyclin dependent kinase-complexes that prevent progression into the next stage of the cell cycle (Levine, 1997). Additionally p53 triggers apoptosis through the intrinsic pathway mediated by Bax and Bak as well as the extrinsic pathway via Fas action. Through different downstream pathways these proteins lead to the release of cytochrome C and other intermitochondrial proteins into the cytosol including caspase formation, leading to cellular degradation (Levine, 1997; Levine et al., 2006; Petros et al., 2004; Vousden, 2005). Mutated p53 has a prolonged half-life and its overexpression can be detected as deposits in the cell nucleus (Hinds et al., 1990). Without normal p53 regulation damaged cells are no longer inhibited from progressing through the cell cycle and are not marked for repair, senescence, or apoptosis. Furthermore the damaged DNA leads to additional genetic mutations that perpetuate cancerous gene formation as well as cells that are resistant to treatment. Several studies have found overexpression of p53 throughout the different stages of carcinogenesis to be a risk factor for progression from BE to EAC, however the exact mechanism has not been completely elucidated (Krishnadath et al., 1995; Murray et al., 2006; Ramel et al., 1992). As a clinical prognostic indicator alterations in p53 expression have been found to be predictive of response to chemotherapy and overall survival (Heeren et al., 2004; Madani et al., 2010).

P16, the protein product of the INK4A/CDKN2A gene, is a cyclin dependent kinase inhibitor that has been demonstrated to be mutated in a variety of cancers including EAC. P16 tumor suppression is initiated by cellular stress leading to the binding of p16 to CDK4 and CDK6. CDK4/p16 and CDK6/p16 complexes inhibit formation CDK-cyclin D complexes, leading to the destruction of cyclin D (Diehl and Sherr, 1997; Rocco and Sidransky, 2001). Without cyclin D, p27<sup>KIP1</sup> accumulation occurs, which in turn prevents CDK2/cyclin E and CDK2/cyclin A complexes from phosphorylating the retinoblastoma protein (Rb). The Rb protein is required for activation of the transcriptional complex E2F-DB and subsequent gene transcription. Additionally the Rb-E2F complexes that form serve as inhibitors of transcription. Given this series of steps, proper p16 regulation responds to cell stress by inhibiting transcription, which is required for the cell to continue through the G<sub>1</sub>/S cell cycle checkpoint (Rocco and Sidransky, 2001). Mutation in p16 genes, occur through point mutations, loss of heterozygosity, and/or silencing of the gene through promoter

hypermethylation (Maley et al., 2004; Rocco and Sidransky, 2001). Therefore, loss of p16 activity in the context of DNA damage and cellular stressors, permit the cell to undergo unregulated transcription and proliferation. There exists an increasing amount of evidence showing that p16 inactivation is a critical step in the development of EAC. In fact the most prevalent genetic alteration in BE is the result of INK4A/CDKN2A hypermethylation, which is an early epigenetic change that occurs in the progression from BE to EAC (Bian et al., 2002; Hardie et al., 2005; Powell et al., 2005; Souza et al., 2001; Vieth et al., 2004).

As a tumor suppressor, the adenomatous polyposis coli (APC) protein has been implicated in the development of EAC (Clement et al., 2006a, b). APC is part of the Wnt signaling pathway which modulates the levels of  $\beta$ -catenin, a key protein for cell-cell adhesion and transcription. This pathway is activated by Wnt proteins binding to receptors of the Frizzled transmembrane protein family and LDL-receptor-related protein, which in turn trigger the phosphorylation of Dishevelled protein. The Dishevelled protein blocks the phosphorylating activity of GSK3 $\beta$ , which is a complex composed of APC, Axin and casein kinase 1 (CK1). When activated, GSK3 $\beta$  phosphorylates  $\beta$ -catenin initiating its destruction. When  $\beta$ -catenin is in its unphosphorylated state it translocates to the cell nucleus, binds to DNA-binding proteins TCF/LEF, and activates gene transcription of growth promoting genes myc, COX-2, matrilysin/matrix metalloproteinase 7, and cyclin D (Clement et al., 2006a; Giles et al., 2003; Logan and Nusse, 2004; Rocco and Sidransky, 2001). Therefore loss of APC, as implicated in a number of cancers, may result in increased  $\beta$ -catenin in the nucleus and uncontrolled cellular proliferation and tumorigenesis (Bian et al., 2000; Clement et al., 2006a; Logan and Nusse, 2004; Trigg, 1998). Furthermore, it has been demonstrated that APC is involved with microtubule function. Without functional APC there is an increase in abnormal mitotic spindles and subsequently chromosomal defects. Several studies have demonstrated that APC inactivation leads to  $\beta$ -catenin accumulation in EAC, but is not necessary or sufficient for activation of the Wnt pathway since  $\beta$ -catenin accumulates without APC inactivation (Clement et al., 2006a). Despite this patients with APC gene hypermethylation in BE samples were more likely to progress to EAC and several studies have shown that all EACs have APC promoter methylation (Wang et al., 2009b). Therefore while the exact mechanism by which loss of APC leads to EAC has not been completely elucidated, its detection might provide prognostic value in assessing the progression from BE to EAC.

Cyclins and cyclin dependent kinases (CDKs) are integral parts of the cell cycle regulation control system. Cyclins bind to CDKs and lead to phosphorylation of proteins necessary for progression through the cell cycle (Stamatakis et al., 2010). In addition to these regulators, p21 acts as a cyclin dependent kinase inhibitor in tumor suppression as well as a possible oncogene, inhibiting apoptosis and promoting proliferation (Abbas and Dutta, 2009; Gartel, 2006). Derangements in the function of these proteins have been implicated in nearly all tumors and their involvement in the progression to BE and EAC is an area of active investigation. Cyclin D1 regulates cell cycle activity by forming a complex with CDK4/6 and controlling activity through G<sub>1</sub>. Once bound, cyclin D1/CDK 4/6 complexes phosphorylate the retinoblastoma protein (Rb), deactivating it and activating E2F transcription complex. E2F leads to the transcription of genes required for transition through G<sub>1</sub> (Shapiro and Harper, 1999; Traganos, 2004). One of the genes transcribed when E2F is activated, cyclin E binds CDK2 leading to phosphorylation of downstream targets that are necessary for replication initiation, histone synthesis, and replication of

centrosomes. This allows transition through G<sub>1</sub> checkpoint to the S phase (Ma et al., 2000). Additionally, cyclin E/CDK2 complexes further phosphorylate Rb leading to additional transcriptional activity (Fu et al., 2004). P21 has been described initially in the tumor suppression cascade of p53 as described above, however new research suggests that it may also have the opposite action as an oncogene promoting tumorigenesis (Gartel, 2006).

Cyclin D1 has been implicated in a number of cancers and is overexpressed due to derangements that include chromosomal translocations, gene amplification and anomalies in proper intercellular trafficking and proteolysis (Kim and Diehl, 2009; Stamatakis et al., 2010). When overexpressed cyclin D1 leads to tumor formation through several mechanisms. First, high levels of cyclin D1 lead to increased activation of CDK4/6 leading to increased proliferation. Second, cyclin D1/CDK complexes inhibit p21 and p27 activity, two CDK inhibitors, and therefore with abnormal levels of p21/p27 inhibition there is decreased inhibitory control over the cell cycle (Cheng et al., 1998). Third cyclin D1 also has non-CDK dependent actions including increasing estrogen receptor transcription (Neuman et al., 1997) as well as abnormalities in the repression of PPAR $\gamma$ , a transcriptional protein modulated by abnormal binding of HDAC by cyclin D1 (Fu et al., 2005). In addition cyclin D1 has been reported to lead to increased expression of fibroblast growth factor 1 (Tashiro et al., 2007) as well as increased production of reactive oxygen species (ROS) leading to metastasis of tumors (Stamatakis et al., 2010). Although the exact mechanism of action in EAC has not been completely elucidated, studies have demonstrated cyclin D1 overexpression in BE and early stages of tumorigenesis (Arber et al., 1996; Bani-Hani et al., 2000).

Aberration in cyclin E activity, from gene amplification (Marone et al., 1998; Stamatakis et al., 2010) or defective degradation (Buckley et al., 1993), leads to constitutive expression and increased activity of the protein, which results in increased cellular proliferation (Stamatakis et al., 2010). Increased levels of cyclin E lead to increased activity of CDK2, subsequent activation of transcription proteins, as well as increased phosphorylation of Rb. Given the pathway described above, these anomalies may all lead to deregulated progression into the S phase and consequently amplified proliferation. This has been noted as a shortened G<sub>1</sub> phase, decreased cell size due to decreased time for growth, and decreased requirement for proper environmental factors necessary for replication (Sala et al., 1997; Stamatakis et al., 2010). The relevance of cyclin E and its deregulation in the development of EAC is currently unclear.

Although P21, a cyclin dependent kinase inhibitor, was thought to solely act in the tumor suppression cascade of p53 as described above, recent evidence suggests that it also has the opposite role as an oncogene, inhibiting apoptosis and promoting cellular proliferation (Abbas and Dutta, 2009; Gartel, 2006; Roninson, 2002). In the tumor suppression cascade of p53, loss of p21 inhibition of cyclin-CDK complexes, especially CDK2, in cells with DNA damage may lead to uncontrolled progression through the cell cycle and carcinogenesis (Abbas and Dutta, 2009). Additionally, it is hypothesized that p21 contains antiapoptotic activity and therefore, when overexpressed, damaged cells avoid degradation and proliferate to form tumors (Roninson, 2002). P21 might also promote cyclin D1 accumulation in the nucleus, therefore avoiding destruction, and facilitating binding with CDK4/6, leading to increased transcription (LaBaer et al., 1997; Liu et al., 2007). P21 has not been extensively studied in BE and EAC, but some evidence suggests that similar to p53 changes



in the expression pattern of p21 may lead to a better response to treatment in these patients (Heeren et al., 2004).

In addition to the main regulatory proteins described above, several other proteins have been implicated in carcinogenesis and may have a role in BE and EAC. Apoptosis is a key component of normal cellular functioning that serves to limit cells with DNA damage by triggering them to self destruct. Two proteins have been implicated in cells evading proper apoptotic pathways, cyclooxygenase 2 (COX-2) and B cell lymphoma 2 protein.

While COX-2 is not constitutively expressed in all tissues, it is found in response to inflammation or mitogenic stimuli ultimately leading to increased production of prostaglandins (PGs) (Konturek et al., 2005). Recent evidence has demonstrated that COX-2 expression increases with worsening grades of dysplasia in esophageal carcinogenesis (Cheong et al., 2003; Konturek et al., 2004) and that the increased COX-2 expression in the esophageal epithelium might be secondary to gastric acid and bile exposure (Shirvani et al., 2000). The mechanisms by which COX-2 overexpression leads to the development of EAC is related to COX-2 derived PGs' actions. These prostaglandins are involved in evasion of apoptosis by inhibiting release of cytochrome c, decreasing activation of caspase-9 and -3, increasing activity of bcl-2 (Wang et al., 2005) and blocking Fas mediated cell death (Nzeako et al., 2002). BE cells with overexpression of COX-2 have lower rates of apoptosis (Wilson et al., 1998). Additionally, COX-2 produced PGs have been implicated in invasion and metastasis, theorized to be due to increased metalloproteinase-2 activity (Nzeako et al., 2002). Furthermore, COX-2 derived PGs have been demonstrated to lead to cell proliferation through stimulation of epidermal growth factor receptors (Baatar et al., 2002), angiogenesis by increasing VEGF levels (Wang and DuBois, 2004); (Shiff et al., 2003) and inhibition of immune anti-tumor responses by hampering the activity of natural killer cells, macrophages, and dendritic cells, and decreasing production of Th1 cytokines. Given these mechanisms, it is not surprising that multiple studies have demonstrated a decreased risk of progression to cancer (Buttar et al., 2002), and decreased cell proliferation in BE epithelium when treated with COX inhibitors (Kaur et al., 2002).

B cell lymphoma 2 protein (Bcl-2) is an integral part in regulation of cell survival by inhibiting apoptosis. Overexpression of Bcl-2 has been documented in a number of cancers as well as all phases of reflux-associated esophageal carcinogenesis including esophagitis, nondysplastic BE, dysplastic BE, and EAC (Metzger et al., 2004). Overexpression of Bcl-2 leads to inhibition of apoptosis early in carcinogenesis leading to decreased cell death and elongated cell lifespan (Metzger et al., 2004) (Lehrbach et al., 2009) (Thomadaki and Scorilas, 2006). The extended cell survival is hypothesized to allow for accumulation of oncogenic mutations leading to carcinogenesis (Zhivotovsky and Orrenius, 2006). Bcl-2 overexpression leads to apoptotic evasion by inhibiting mitochondrial release of cytochrome c and eventual caspase formation (Thomadaki and Scorilas, 2006). In fact, cells with elevated Bcl-2 production are more resistant to chemotherapy and radiation treatment (Thomadaki and Scorilas, 2006). However, its exact role in EAC development has not been extensively studied.

In order for tumors to survive, invade, and metastasize there are a number of proteins involved including Tissue inhibitors of metalloproteinase (TIMPs). These classes of proteins have distinct roles in this aspect of tumorigenesis and have been associated with BE and EAC.

TIMPs are enzymes that regulate the production and actions of metalloproteinases (MMPs), which are responsible for turnover and remodeling of the extracellular matrix and cell signaling, in addition to non-MMP dependent actions. TIMPs have been evaluated in a number of cancers and have demonstrated multi-factorial and contradictory roles in cancer, with both overexpression and silencing observed. Furthermore, it is theorized that TIMPs exhibit different actions depending on the level of expression. Mechanisms by which TIMPs are involved in carcinogenesis include apoptosis and cell proliferation, angiogenesis, and metastasis and cell adhesion (Bourboulia and Stetler-Stevenson, 2010; Cruz-Munoz and Khokha, 2008; Jiang et al., 2002; Sun, 2010). In cancer cell lines, TIMP-1 enhanced cell survival and growth by increasing expression of IL-10 and anti-apoptotic protein bcl-xl (Chirco et al., 2006). TIMPs promote cell proliferation by increasing p65 phosphorylation, which increases NF- $\kappa$ B, a protein that binds gene promoters and leads to cell proliferation. This, however, was observed at late stages of tumor growth while upregulation of NF- $\kappa$ B has also demonstrated slowing tumor growth in its early stages. The exact role in proliferation seems to depend on the stage of tumorigenesis (Sun, 2010). TIMPs are thought to enhance angiogenesis by increasing vascular endothelial growth factor (VEGF) production and inhibiting MMPs, which, produce angiogenesis inhibitors, endostatin and angiostatin (Jiang et al., 2002). As well, TIMP-2, which normally blocks microvascular endothelial cell growth in response to pro-angiogenic factors like FGF-2 or VEGF A is silenced through promoter hypermethylation in a number of cancers (Sun, 2010). Lastly, TIMPs normally inhibit cell adhesion and metastasis by blocking the breakdown of the extracellular matrix through inhibition of MMPs. However, in cancers there is a disruption of the balance between MMPs and TIMPs, with possible elevated levels of MMPs and increased disruption of the ECM and cell-cell adhesion leading to increased cell motility (Bourboulia and Stetler-Stevenson, 2010; Cruz-Munoz and Khokha, 2008). Given these mechanisms in a number of cancer, the exact role of TIMPs in esophageal adenocarcinoma development from BE has not been elucidated.

Lastly the plasminogen activating system, which includes urokinase-type plasminogen activator (uPA), has been investigated for its prognostic value in BE. In normal physiologic states, this system regulates the fibrinolytic system, however it has been implicated in a variety of pathologic states including tumor cell proliferation, apoptosis, cell migration and invasion, and angiogenesis (Laufs et al., 2006; McMahon and Kwaan, 2008). uPA, a serine protease has been demonstrated to be expressed in tumor cells, and is considered the most active component of this system. uPA is involved in cell proliferation, apoptosis, and angiogenesis through interaction with its cellular receptor uPAR and the epidermal growth factor receptor (EGFR), which stimulates cell growth through a cascade of intracellular mechanisms (McMahon and Kwaan, 2008). uPA is believed to assist in tumor cell migration and invasion by converting plasminogen to plasmin, which activates MMPs, which degrade ECM elements such as vitronectin, laminin, and type IV collagen, leading to altered cell adhesion, shape, and migration (Laufs et al., 2006; McMahon and Kwaan, 2008). Angiogenesis is altered by uPA by affecting endothelial cell proliferation leading to angiogenesis and by activating kringle structures, which inhibit microvascular endothelial cell proliferation through changes in angiostatin levels therefore inhibiting angiogenesis.

Several proteins have been implicated in tumorigenesis and may have prognostic implications in EAC including cadherins and Ki-67. The mechanism by which these proteins promote oncogenesis, provide opportunity for further investigation.

### 3. Growth factors

The mechanisms that promote evolution of BE to EAC are largely unknown. As derangements in the cell cycle are believed to be involved in carcinogenesis and the development of uncontrolled cellular replication, growth factors have been the focus of investigations in the neoplastic progression of BE. Three such factors that have been recognized as promoting growth in BE are: epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and transforming growth factor beta (TGF- $\beta$ ).

The epidermal growth factor receptor (EGFR) family of receptors has been studied as a potential biomarker in the progression of BE to EAC. This family of tyrosine kinase receptors initiates a signal transduction cascade that modulates cell proliferation, differentiation, adhesion, and migration (Yarden, 2001). EGFR is a transmembrane receptor that enables signals to be transmitted across the plasma membrane, affecting gene expression and a multitude of cellular responses. Overexpression of this receptor has been shown to occur in several malignancies, including EAC (Wang et al., 2007).

The correlation of EGFR expression with early neoplastic progression of BE has not been completely elucidated. Using PCR to evaluate gene expression, amplification of the EGFR locus was demonstrated in EAC without concomitant elevated expression in high-grade dysplasia with BE (Miller et al., 2003). However, another study did show gene locus amplification of EGFR in both BE associated high-grade dysplasia and EAC (Rygiel et al., 2008). There is also the possibility of a certain EGF polymorphism leading to an increased risk of EAC, as the specific EGFA61G G/G genotype has been shown to confer such a risk (Lanuti et al., 2008).

While there is evidence linking EGFR gene expression with EAC, until recently there was only limited evidence of this expression in regards to protein abundance (Li et al., 2006; Wilkinson et al., 2004). Importantly, for EGFR to be considered a useful biomarker of histological progression of BE, it would increase in progression during neoplastic transformation. In addition, it would also be expressed on the luminal epithelial surface so as to be readily visualized during endoscopy, and be available for biopsy targeting. One recent study evaluated this potential dual role for EGFR using tissue microarray technology, exploring the possibility of EGFR as a relevant biomarker to monitor histological progression, and ultimately to allow for biopsy targeting of abnormal tissue (Cronin et al., 2011). The study showed a stepwise increase in EGFR abundance in BE, high-grade dysplasia, and EAC. As EGFR is a transmembrane protein expressed on the luminal esophageal surface, it also has potential for sampling during endoscopy.

VEGF is another growth marker that performs an important role in tumor formation. Increased vascularity is associated with a poor prognosis in several human malignancies, and VEGF is important for angiogenesis in neoplastic progression. By using immunohistochemistry to examine VEGF expression, one study specifically studied vascularization in both BE and associated EAC (Couvelard et al., 2000). An increase in angiogenesis was found in precancerous lesions, and VEGF expression correlated with the increase in vascularization. However VEGF in the study had no prognostic significance.

As it is accepted that BE develops from esophageal mucosal injury incurred after acid and bile reflux, a study looked at VEGF expression in a bile acid environment. By quantitative



PCR, VEGF expression to increased after exposure to certain bile acids (Burnat et al., 2007). Another study looked at the possibility of an “angiogenic switch” in the transition from metaplasia to dysplasia to carcinoma in BE (Mobius et al., 2003). This study showed that VEGF expression increased during the sequence of metaplasia to advanced carcinoma. More specifically, the data suggested that the related impact on neovascularization occurred early on in the course of this transformation, as the only true significant difference in VEGF expression occurred between Barrett’s metaplasia and high-grade dysplasia. This entailed that the importance of VEGF and related angiogenesis in the progression to EAC may occur before actual tumor growth.

TGF- $\beta$  is a growth factor predominantly involved in cellular proliferation and differentiation, and several studies suggest that loss of TGF- $\beta$  signaling is an important factor in BE-related EAC. Smad4, a protein that is part of a TGF- $\beta$  mediated complex important in downstream gene activation, has been shown to potentially have a dual importance in the neoplastic progression of BE. In response to TGF- $\beta$ , a majority of EAC cell lines in one study failed to growth arrest, and specific modulation of Smad4 was inhibited. However, the cell lines also upgraded the expression of certain proteases that led to a more invasive cell phenotype, suggesting a dual role for TGF- $\beta$  (Onwuegbusi et al., 2007). By PCR, FISH, and sequencing, Smad4 expression has also been shown to be progressively reduced in the metaplasia to dysplasia to adenocarcinoma sequence (Onwuegbusi et al., 2006).

Other evidence propose additional roles for the downstream TGF- $\beta$  pathway and its importance in the neoplastic progression of BE. One study showed that hypermethylation and inactivation of RUNX3, a target gene of TGF- $\beta$ , is associated with the progression of BE to dysplasia and ultimately adenocarcinoma (Schulmann et al., 2005). TGF- $\beta$  also has known significance in the epithelial to mesenchymal transition, which promotes cellular motility, invasion, and cytoskeletal rearrangement in a range of tumor cells. One study looked specifically at the ability of TGF- $\beta$  to induce esophageal to mesenchymal transition in esophageal cell lines in vitro. The data from this study suggested a role for this transition in EAC, as TGF- $\beta$  induced alterations in aggregation and invasion, and thus a more invasive phenotype (Rees et al., 2006).

There has been clear value in the investigation of EGF, VEGF, and TGF- $\beta$  as performing critical roles in the progression of BE to EAC. More examination is needed to clarify the independent function of these growth factors and their downstream proteins in the progression of this disease.

#### 4. Adiposity and adipokines

Obesity, as defined by a body mass index (BMI)  $>30$ , has been increasing steadily over the past twenty years and has become an epidemic in the US. In 1989 the prevalence of obesity among adults did not surpass 15% in any state. By contrast in 2009, the prevalence of obesity among adults is greater than 15% in every state [Ref: <http://www.cdc.gov/obesity/data/trends.html#State>]. It is estimated that 68% of adults twenty years of age or older in the US are either overweight or obese and nearly 34% of these are obese (Flegal et al., 2010). Obesity is associated not only with metabolic disorders such as diabetes (Bray, 1992; Pontiroli and Galli, 1998; Scott et al., 1997) but also with neoplastic conditions such as EAC (Chow et al., 1998; Dvoyrin et al., 2011; Lagergren et al., 1999b). Elucidating the

biomolecular mechanisms that link adiposity with the development of GERD, BE, and EAC is an active area of investigation. Such information could provide novel targets for the treatment of conditions along the GERD spectrum.

Increased adiposity, defined by BMI or waist circumference, has been shown to be an independent risk factor for GERD epidemiologically, while being consistently linked to reflux symptoms as well as mucosal injury (Hampel et al., 2005). Increasing BMI and waist circumference separately lead to an increased frequency of GERD symptoms, esophageal acid exposure, (El-Serag et al., 2007; El-Serag et al., 2005; Locke et al., 1999; Murray et al., 2003) and reflux related hospitalizations (Ruhl and Everhart, 1999). In fact, those with a BMI greater than 30 kg/m<sup>2</sup> have been shown to be approximately three times more likely to develop GERD symptoms at least once per week (Locke et al., 1999; Murray et al., 2003). Even among those who are morbidly obese, those with a higher BMI have a higher percentage of time during which the esophageal pH is less than four (Fisher et al., 1999).

Obesity has also been linked to complications of GERD including the development of BE and EAC. These develop due to an imbalance between injurious elements and esophageal protective mechanisms (Vaezi and Richter, 1996). It is estimated that obesity leads to a two and a half fold increased risk of BE. Furthermore Stein and colleagues concluded that for every 10-pound increase in weight the risk of BE increases by 10%, and for each five point increase in BMI the risk increases by 35% (Stein et al., 2005). Recent evidence also suggests that abdominal obesity, specifically visceral fat, is a stronger risk factor for BE than BMI (Corley et al., 2007; Edelstein et al., 2007). In terms of EAC, a linear relationship with obesity seems to exist as higher BMI levels are associated with an increased risk of the malignancy (Brown et al., 1995; Chow et al., 1998; Lagergren et al., 1999b; Wu et al., 2001).

Several mechanisms have been proposed to explain the association between the level of adiposity and the development of GERD. The "transmitted pressure" hypothesis suggests that direct mechanical pressure from the large abdominal panus can lead to an increased intragastric pressure, which when relayed to the lower esophageal sphincter, can result in reflux by way of non-swallow induced transient relaxation (El-Serag, 2008; El-Serag et al., 2006; Lambert et al., 2005; Mercer et al., 1985; Pandolfino et al., 2006). Pandolfino et al. demonstrated a multi-faceted mechanism by which fat, especially visceral adiposity, leads to reflux. Utilizing high-resolution manometry, this study demonstrated that for every inch of increased waist circumference, there is a significant increase in intragastric and intraesophageal pressures, 0.4 mm Hg and 0.1 mm Hg, respectively. Additionally both the mean gastroesophageal pressure gradient (GEPG) as well as the disruption of the esophagogastric junction (EGJ), measured as separation of the lower esophageal sphincter and extrinsic crural diaphragm, were higher among obese patients. Furthermore, EGJ disruption allows for the development of a hiatal hernia. Finally, patients with GERD symptoms when compared to non-GERD patients had increased GEPG and EGJ disruption. Given these pressure morphologies among overweight and obese patients, it appears that increasing panus size creates a pathophysiologic mechanism allowing for the flow of gastric contents into the esophagus (Pandolfino et al., 2006).

As an active endocrine organ, adipose tissue is also important metabolically, and produces adipocytokines such as leptin, and adiponectin. In addition while the gastric epithelium produces acid, it is also the primary source for the adipokine ghrelin. Evidence suggests that

these hormones are associated mechanistically in the development of BE and esophageal adenocarcinoma.

Leptin, a proteohormone produced mostly in proportion to the amount of white adipose tissue, is a diverse hormone involved in energy homeostasis and satiety management (Housa et al., 2006). Leptin is also produced by the gastric epithelium and we have found that fundic levels to be significantly associated with risk of BE (Francois et al., 2008). In esophageal cell lines, leptin has been shown to have actions as a growth factor leading to increased proliferation (Beales and Ogunwobi, 2007; Lipetz, 1984; Somasundar et al., 2003) and inhibition of apoptosis, which may predispose to an increased risk of BE and esophageal adenocarcinoma (Beales and Ogunwobi, 2007). These effects have been demonstrated to be synergistic when leptin is combined with acid pulses (Beales and Ogunwobi, 2007). The most current research has demonstrated a complex signaling pathway by which leptin causes these aberrations, however the exact mechanism has not been completely elucidated. It is postulated that leptin stimulates a transmembrane leptin receptor, which activates both P38 MAP kinase and janus kinase JAK2 pathways. JAK2 also activates P38 MAP kinase, as well as, extracellular signal related kinase (ERK) and Akt, which all increase COX-2 mRNA levels. Upregulation of COX-2 leads to increased PGE-2 production, which subsequently activates an EP-4 receptor. The EP-4 receptor then causes activation of PKC, src, and MMPs, which increase cleavage and extracellular shedding of EGFR ligands, HB-EGF and TGF $\alpha$ . These transactivate an epidermal growth factor receptor (EGFR) (Beales and Ogunwobi, 2007; Ogunwobi et al., 2006; Ogunwobi and Beales, 2008b). Ultimately, C-jun NH<sub>2</sub> terminal kinase (JNK) is then activated which leads to the inhibition of apoptosis and increased proliferation. It is by this pathway which leptin may predispose obese patients to an increased risk of EAC (Beales and Ogunwobi, 2007; Ogunwobi et al., 2006).

Adiponectin is produced solely by white adipose tissue and has been shown to have anti-atherogenic, anti-inflammatory, and anti-diabetic actions (Housa et al., 2006; Nishida et al., 2007). It is a protein found in multiple isoforms, high molecular weight (HMW), medium molecular weight (MMW), and low molecular weight (LMW) (Suzuki et al., 2007). Unlike leptin, adiponectin levels are decreased in obesity, and low levels of adiponectin have been associated with an increased risk of BE and EAC (Rubenstein et al., 2008; Yildirim et al., 2009). Its role in the development of cancer is believed to be protective through anti-proliferative (Ogunwobi and Beales, 2008a) and pro-apoptotic effects (Konturek et al., 2008). Adiponectin leads to a dose-dependent increase in the rate of apoptosis in esophageal cancer cell lines, which can be explained by a dose dependent increase and decrease in mRNA and protein expression of pro-apoptotic BAX and anti-apoptotic Bcl-2, respectively (Konturek et al., 2008). Its anti-proliferative effects may be mediated through its actions on adiponectin receptor-1 as well as activation of adenosine monophosphate activated protein kinase and serine/threonine phosphatases, which ultimately lead to modulation of p53 and p21 (Ogunwobi and Beales, 2008a); (Rubenstein et al., 2009). More recent evidence suggests unique roles of each adiponectin multimer in the development of BE. High levels of LMW adiponectin have been shown to be associated with a decreased risk of BE, and conversely, high levels of HMW adiponectin are associated with an increased risk (Rubenstein et al., 2009). LMW adiponectin may prevent the inflammatory reaction of esophageal mucosa by suppressing the release of pro-inflammatory interleukin-6 and increasing release of anti-inflammatory interleukin-10. Therefore, low levels of LMW adiponectin might permit an aberrant response to reflux thus leading to metaplastic changes (Schober et al., 2007). Other

possible mechanisms of adiponectin that have been postulated include suppression of extracellular signal related kinases 1 and 2, which when exuberantly activated lead to increased proliferation and decreased apoptosis, and possibly an exacerbated pathologic response to reflux (Rubenstein et al., 2008).

Lastly, ghrelin is a hormone that is produced primarily in the fundus of the stomach, (Kojima and Kangawa, 2005) and has a variety of roles which include stimulation of appetite as well as gastric acid secretion (Masuda et al., 2000; Wren et al., 2001). Ghrelin levels have been shown to be inversely related to adiposity and EAC risk (de Martel et al., 2007), but the biomolecular mechanism underlying this association has not been completely elucidated. There may be an anti-inflammatory component to the mechanism, as evidence suggests that ghrelin inhibits the increased production of COX-2 and interleukin-1 $\beta$  (IL-1 $\beta$ ) by tumor necrosis factor alpha (TNF $\alpha$ ) (Konturek et al., 2008). In addition, ghrelin stimulates gastric motility by stimulating the vagus nerve and myenteric neurons. By shortening gastric emptying time, this decreases the acid exposure of the lower esophageal and potentially decreases the risk of EAC (de Martel et al., 2007).

By further elucidating the mechanisms behind adipocytokines and their relationships to BE and EAC, there may be opportunities for both diagnostic as well as therapeutic approaches to these sequelae of GERD.

## 5. Microbiome and cancer

The human body is inhabited by ten times more bacteria than the number of human cells in the body (Savage, 1977). The bacteria form ecological communities on every external (skin) and internal (mucosal) surfaces of our body. Bacteria in the communities collectively are termed by Joshua Lederberg as "microbiome" (Lederberg and McCray, 2001). The host relationship with the microbiome can be commensal, symbiotic, and pathogenic. The general concept is that the host provides a nutrient-rich habitat, while the bacteria play important roles in the development of the mucosal immune system, the maintenance of a physiological environment, the provision of essential nutrients, and the prevention of colonization by pathogenic bacteria (Cunningham-Rundles et al., 2002; Eckburg et al., 2005). On the other hand, certain members of the human microbiome play a pathogenic role, as illustrated by traditional medical microbiology built upon concepts of infectious diseases in which a pathogen can often be identified and pathogenesis explained by toxins or virulent factors produced by the pathogen. These concepts have clearly demonstrated their usefulness in the identification of etiologic agents of a number of infectious diseases.

More recently, the concept that the microbiome is essential for the development of inflammation-induced carcinoma has emerged from studies of well-known colonic microbiome (Yang and Pei, 2006). In the TCR $\beta$ /p53, IL-10 and Gpx1/Gpx2 knockout mouse colitis models that mimic the development of adenocarcinoma in ulcerative colitis, carcinoma develops in conventional mice but not in mice raised under germ-free housing conditions (Balish and Warner, 2002; Chu et al., 2004; Kado et al., 2001). In IL-10, HLA-B27/ $\beta$ 2m, and TCR $\alpha$  knockout mice, the colonic microbiome is also a prerequisite for the development of inflammation in the colon (Kawaguchi-Miyashita et al., 2001; Rath et al., 1996; Sellon et al., 1998; Song et al., 1999). Although intestinal bacteria are an important factor in the inflammation and tumorigenesis, their precise role remains elusive. One theory



is that both “protective” species and “harmful” species exist within the normal enteric microbiome. A healthy balance between these two populations in a normal host might be detrimental for an inflammation-prone host. Alternatively, a breakdown in the balance between the two populations, termed “dysbiosis”, could by itself promote inflammation in a normal host. Chronic inflammation could be carcinogenic. Although resident enteric bacteria are necessary for the development of spontaneous colitis in many rodent models, not all bacteria have an equivalent capability to induce inflammation. Germ-free IL-10-deficient mice populated with bacterial strains, including *Bacteroides vulgatus*, *Clostridium sordellii*, *Streptococcus viridans*, *Escherichia coli*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, and *Lactobacillus lactis* do not exhibit significant colitis (Balish and Warner, 2002; Sellon et al., 1998; Sydora et al., 2005). In contrast, *Citrobacter rodentium*, *Helicobacter hepaticus*, *Enterococcus faecalis* are examples of conditional cancer-causing bacteria that alone do not cause cancer, but are carcinogenic in certain genetically-engineered immunodeficient mice (Balish and Warner, 2002; Barthold and Jonas, 1977; Engle et al., 2002; Erdman et al., 2003; Newman et al., 2001; Sellon et al., 1998; Sydora et al., 2005; Ward et al., 1994).

The observations in rodent models which depict the role of the microbiome in tumorigenesis raise some interesting possibilities in relation to human cancers. Although there is no established bacterial pathogen for human colorectal cancer, unusual infections might precede the clinical diagnosis of cancer. A significant proportion (13%) of patients with *Streptococcus bovis* bacteremia have colon cancer (Panwalker, 1988). In some cases, the bacteremia occur months or years before the cancer is diagnosed. *Clostridium septicum* infections are rare, but are often (81%) associated with malignancy including colon cancer (34%) (Beebe and Koneman, 1995; Kornbluth et al., 1989).

The lessons learned from investigations of the colonic microbiome could serve as a general guide to studies of the etiology and pathogenesis of chronic inflammatory diseases and related cancers in other sites of the gastrointestinal tract. These include conditions such as reflux esophagitis and esophageal adenocarcinoma, *Helicobacter* gastritis and gastric adenocarcinoma and lymphoma, as well as inflammatory bowel diseases (IBD) and colorectal cancer.

### **5.1 *Helicobacter pylori* as a protective marker for esophageal adenocarcinoma**

*Helicobacter pylori* is the most established bacterial cause of human cancer. In particular, *H. pylori* causes gastric adenocarcinoma, the fourth most common cancer and second leading cause of cancer-related death in the world (Ferlay et al., 2010; Parkin et al., 2005; Peek and Blaser, 2002). It is a Gram-negative, microaerophilic bacterium that colonizes the stomach of at least half the world's population (Pounder and Ng, 1995). *H. pylori* causes chronic active gastritis and the colonization and inflammation may persist in the stomach for life if not treated (Marshall and Warren, 1984). Although most individuals infected by *H. pylori* are asymptomatic, despite having chronic gastritis, approximately 10-20% of the patients will develop gastric and duodenal ulcers (Kusters et al., 2006). *H. pylori* infection is also associated with a 1-2% lifetime risk of stomach cancer and a less than 1% risk of gastric MALT lymphoma (Kusters et al., 2006). The risk of non-cardia gastric cancer was nearly six times higher for *H. pylori*-infected people than for uninfected people (Webb et al., 2001). In 1994, the International Agency for Research on Cancer (IARC) classified *H. pylori* as a group 1 carcinogen (IARC, 1994). *H. pylori* infection causes gastric cancer through interaction

between bacterial virulent factors and human genes/pathways. *H. pylori* may directly damage the gastric mucosa by bacterial products such as ammonia, phospholipases, and toxins and causes mutations by promoting persistent tissue repair and cellular proliferation. Secondary response from the host, such as releasing free radicals, reactive oxygen metabolites, and cytokines can further damage the gastric mucosa (Bechi et al., 1996). *H. pylori* strains exhibit extensive genetic diversity and strain-specific proteins augment the risk for malignancy (Polk and Peek, 2010). Based on the presence or absence of cytotoxin-associated gene A (cagA), *H. pylori* can be divided into cagA-positive and cagA-negative strains (Covacci et al., 1993; Tummuru et al., 1993). Infection with cagA-positive *H. pylori* strains has been associated with more severe mucosal inflammation, atrophic gastritis and increased risk for the development of gastric carcinoma (Blaser et al., 1995; Kuipers et al., 1995; Parsonnet et al., 1997). There is a twofold increase in the risk of gastric carcinoma associated with cagA-positive strains compared to cagA-negative strains (Huang et al., 2003). *H. pylori* also augment its carcinogenicity by expression of active vacuolating cytotoxin (VacA) as well as blood-group antigen-binding adhesin (BabA1) (Figueiredo et al., 2002; Louw et al., 2001; Miehlke et al., 2001; Rhead et al., 2007). Eradication of *H. pylori* leads to a modest reduction in gastric cancer risk (Fuccio et al., 2009). On the host side, polymorphisms that are associated with increased expression of IL-1 $\beta$  and TNF $\alpha$  also increases the risk of gastric cancer (El-Omar et al., 2003).

Paradoxically, *H. pylori* infection might prevent against the development of EAC. In the majority of studies comparing the rates of *H. pylori* infection in patients with reflux disorders, *H. pylori* infection is associated with a reduced risk of BE and EAC (Islami and Kamangar, 2008; Rokkas et al., 2007; Ronkainen et al., 2005; Sorberg et al., 2003; Wang et al., 2009a). In particular, *H. pylori* infection is inversely associated with risk for EAC with an odds ratio (95% CI) of 0.56 (0.46-0.68), as shown in a meta analysis of 19 case control studies (Islami and Kamangar, 2008). The risk reduction is due to infection with CagA-positive strains (OR, 0.41; 95% CI, 0.28-0.62) as CagA-negative strains are not protective (OR, 1.08; 95% CI, 0.76-1.53). *H. pylori* infection and CagA-positive strains are also associated with a reduced risk for EAC in another meta analysis (Rokkas et al., 2007). Similarly, BE is inversely correlated with both the *H. pylori* prevalence (OR, 0.64; 95% CI, 0.43-0.94; P = .025) and the prevalence of *H. pylori* cagA-positive strain (OR, 0.39; 95% CI, 0.21-0.76; P = .005) (Rokkas et al., 2007). More recent meta analysis found that the prevalence of *H. pylori* infection is significantly lower in BE than in endoscopically normal healthy controls (23.1% vs. 42.7%, OR=0.50, 95% CI 0.27-0.93, P=0.03) but significantly higher in BE patients in studies using healthy blood donors as "normal controls" (71.2% vs. 48.1%, OR=2.21, 95% CI 1.07-4.55). The discrepancy appears to be due to difference in study designs (Wang et al., 2009a). The healthy blood donors are not appropriate controls because: i). the prevalence of *H. pylori* infection in the blood donors is different from that in the general population (Sorberg et al., 2003); ii). Some of the "healthy" blood donors may actually have unrecognized BE since they are not examined by endoscopy. BE is present in 1.6% of the general Swedish population (Ronkainen et al., 2005).

Although no simple theory can explain why *H. pylori* infection reduces the risk of BE and EAC, there are several plausible hypotheses. First, *H. pylori* infection may decrease the tissue damage caused by gastroesophageal reflux by lessening the acidity in the refluxate (Ye et al., 2004). The decrease in gastric acidity could be a result of chronic inflammation and atrophic gastritis associated with *H. pylori* infection as well as the action of bacterial urease that

produces ammonia and neutralize gastric acid, independent of gastric atrophy (Richter et al., 1998). This hypothesis is consistent with the observed inverse relationship between indices of *H. pylori* infection and occurrence of gastroesophageal reflux symptoms (Raghunath et al., 2003). However, simple achlorhydria in patients with pernicious anemia does not appear to be sufficient for reduction of the risk for EAC, contradicting this hypothesis (Ye and Nyren, 2003). Second, *H. pylori* infection suppresses levels of ghrelin, a potent appetite stimulant and potential contributor to obesity (Azuma et al., 2002; Jang et al., 2008; Nwokolo et al., 2003; Roper et al., 2008; Tatsuguchi et al., 2004; Thrift et al., 2011). Because obesity increases the risk for GERD, BE and EAC (Cook et al., 2008; Hampel et al., 2005; Kubo and Corley, 2006; Whiteman et al., 2008), it is possible that *H. pylori* infection may decrease the risks for reflux disorders by reducing body weight. Paradoxically, high ghrelin levels (rather than low) reduce the risk for EAC (de Martel et al., 2007). Third, *H. pylori* is capable of inducing esophageal cancer cells to die through apoptosis *in vitro* experiments, which depends on the presence of CagA (Jones et al., 2003). Thus, *H. pylori* might reduce the EAC risk by killing the cancer cells. However, this finding may not be clinically relevant because *H. pylori* does not colonize Barrett's mucosa (Buttar and Wang, 2004). Ultimately the perceived mechanism behind which *H. pylori* infection may protect against BE and EAC remains unclear.

## 5.2 Microbiome alteration as a marker for Barrett's esophagus

The esophagus, as with other luminal organs of the digestive system, represents a suitable environment for bacteria to inhabit. Besides residential bacteria, extraneous bacteria could be introduced into the esophagus by swallowing or by reflux from the stomach. However, compared with the well-studied colonic and oral microbiomes, characterization of the esophageal microbiome has received less attention. Attempts to define the esophageal microbiome have been described in 9 reports (Table 1). Previous culture-based studies suggested that the esophagus is either sterile or contains only few transient bacteria (Gagliardi et al., 1998) but studies (Narikiyo et al., 2004; Pei et al., 2004; Pei et al., 2005; Yang et al., 2009) using cultivation-independent PCR have consistently identified indigenous bacteria associated with mucosal surfaces in tissue biopsies. Furthermore, the bacteria are visible on the mucosal surfaces of the distal esophagus (Figure 1) (Pei et al., 2004). Much of the interest to further study of the microbiome comes from its possible link to esophageal disease.

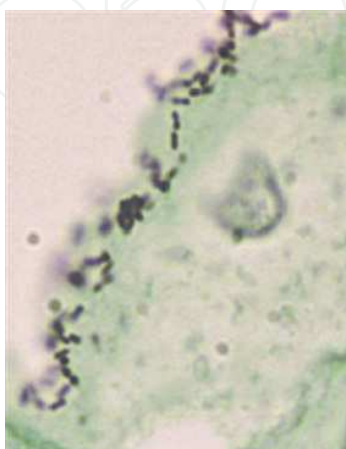


Fig. 1. Visualization of bacteria in the distal esophagus by gram stains in normal.

Category	Culture-based (1981-2007)					Non-culture-based (2004-2009) By Sanger sequencing			
	Lau 1981	Finlay 1982	Mannell 1983	Gagliardi 1998	Macfarlane 2007	Narikiyo 2004	Pei 2004	Pei 2005	Yang 2009
Disease	Cancer	Cancer	Cancer	Normal	BE	Cancer	Normal	RE, BE	RE, BE, Normal
Specimen	Aspirate	Resection	Aspirate	Aspirate	Biopsy Aspirate	Biopsy	Biopsy	Biopsy	Biopsy
No. cases	79	12	101	30	14	20	4	24	34
No. isolates or clones	61	85	377	30	ND	100	900	147	6,800
No. species	14	15	32	11	46	7	95	39	166
Mean species per case	1	6	4	1	ND	≤6	43	ND	24.7
% cases positive for bacteria	64	100	100	67	71	ND	100	100	100
% cases positive for <i>Streptococcus</i>	10	92	ND	ND	50	87	100	ND	100

Table 1. Summary of culture-based and non-culture-based studies on bacterial biota of the esophagus

A recent study of human distal esophagus microbiome (Yang et al., 2009) linked inflammation and BE to the change in the microbiome. The study used 16S rRNA gene survey to characterize the bacterial communities in biopsy samples taken from the distal esophagus of 34 individuals with either normal mucosa (n=12), esophagitis (n=12), or BE (intestinal metaplasia) (n=10). Two hundred 16S rRNA genes were cloned and sequenced from each sample. Overall, the 6800 sequences represented 9 phyla, 70 genera, or 166 species. Firmicutes is the only phylum consistently detected in all 34 samples, whereas the other 8 phyla, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, TM7, Spirochaetes, Cyanobacteria and unclassified bacteria were less common. The samples from healthy subjects were dominated by *streptococci*. On average, 76% of the sequences from healthy esophageal mucosa were categorized to belong to *streptococcal* species and the numbers of some other species were low, but significantly increased in reflux esophagitis and BE.

With an unsupervised approach, samples of the microbiome form two distinct clusters or two microbiome types, type I and II (Figure 2), based on combined genetic distance between samples. Although neither of the two types of microbiome exclusively correlated with the 3 phenotypes, the type I microbiome is more closely associated with normal esophagus (11/12, 91.7%), whereas the type II microbiome is mainly associated with abnormal esophagus (13/22, 59.1%) ( $p=0.0173$  among group comparison), including both esophagitis (7/12, 58.3%,  $OR=15.4$ ) and BE (6/10, 60.0%,  $OR=16.5$ ) (Table 2). The alteration of microbiome from type I to type II in distal esophagus, thus, is associated with host phenotypes and its disease progression.

*Streptococcus* is the most dominant genus in the esophageal microbiome and its relative abundance differs between the two types of microbiome and decreases in disease states. Overall, the 20 type I samples had a mean of 78.8% *Streptococcus* (range, 60.5%–97.0%), whereas the 14 type II samples had a mean of 30.0% (range, 8.0%– 46.5%) ( $P < 1 \times 10^{-10}$ ). The mean of relative abundance of *Streptococcus* in the normal esophagus group (75.9%) was significantly higher than that in the esophagitis (50.5%) and BE (54.1%) groups (Table 3) (Figure 3).



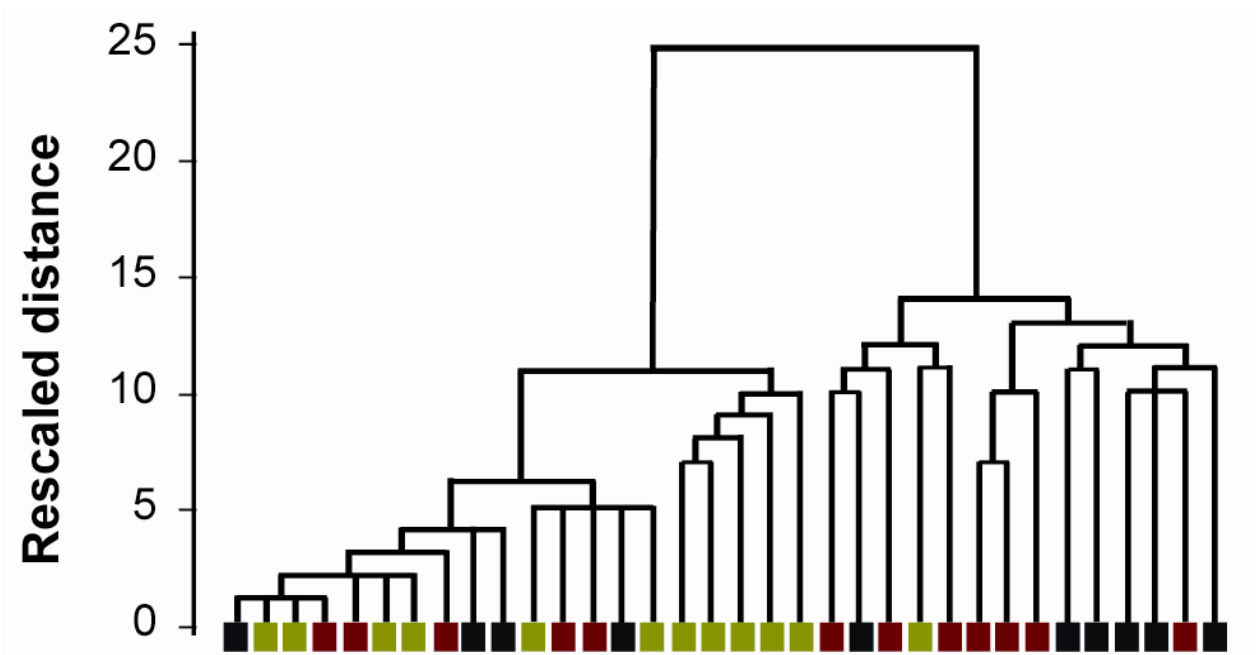


Fig. 2. Typing of esophageal microbiome. Detection of natural microbiome groups by unsupervised cluster analysis. The dendrogram was constructed using the average linkage algorithm and cosine measure of the genetic distance calculated from samples of the microbiome. Samples are represented by colored rectangles (green for normal, red for esophagitis, and black for Barrett’s esophagus).

Omnibus test <sup>A</sup>							
Groups compared		Phenotype					
		Normal		Esophagitis	BE <sup>B</sup>		
Microbiome type	I	11		5		4	
	II	1		7		6	
P value		0.0173					
Follow-up tests <sup>C</sup>							
Groups compared		Phenotype					
		Normal	Esophagitis	Normal	BE	Esophagitis	BE
Microbiome type	I	11	5	11	4	5	4
	II	1	7	1	6	7	6
P value		0.027*		0.020*		1.000	
Odds ratio (95% C.I.)		15.4 (1.5-161.0)		16.5 (1.5-183.1)		1.1 (0.2-5.9)	

<sup>A</sup> The Omnibus test was performed using the two-tailed Fisher-Freeman-Halton 3 × 2 probability test.

<sup>B</sup> BE, Barrett’s esophagus.

<sup>C</sup> The follow-up tests were performed with the two-tailed Fisher exact 2 × 2 probability test. Tests that are statistically different at the false discovery rate < 5% <sup>21</sup> are marked by \*.

Table 2. Association between host phenotypes and microbiome types in the distal esophagus

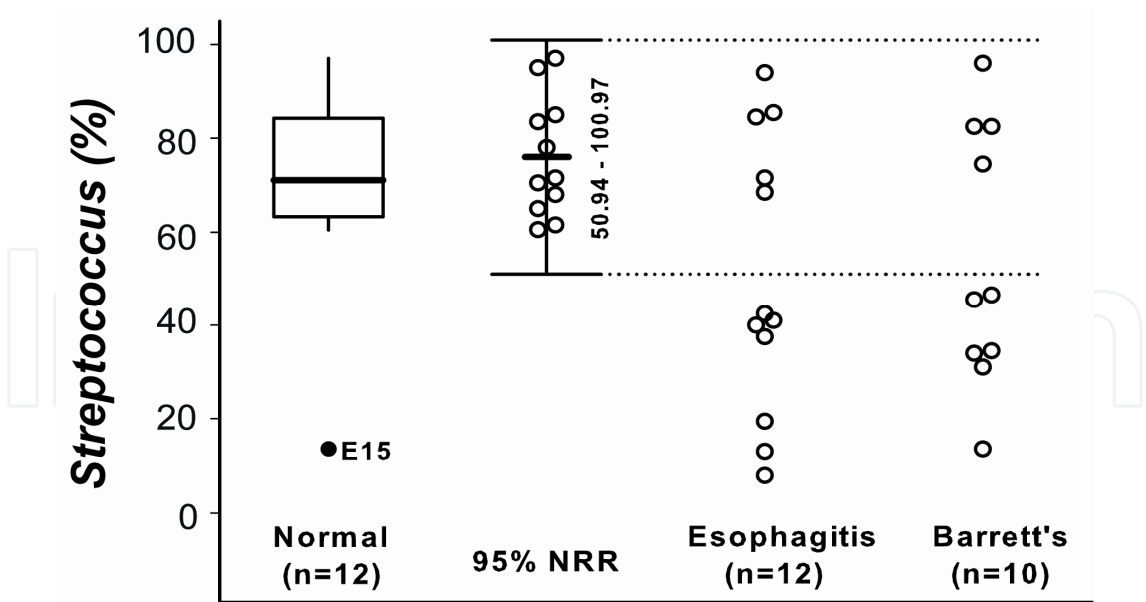


Fig. 3. Taxonomic definition of microbiome types. Classification of microbiome by the relative abundance of *Streptococcus*. An outlier (solid circle) was excluded using a box plot in which the upper whisker length is 1.5\*IQR. The 95% normal reference range (NRR) (mean  $\pm$  1.96 S.D.) was calculated by the relative abundance of *Streptococcus* after excluding the outlier. The dotted line (50.3%) is the upper limit of the 95% normal reference range (NRR), which separates the 34 samples into normal (inside the NRR) and abnormal taxonomic types (outside the NRR).

Omnibus test <sup>a</sup>						
Groups compared	Phenotype					
	Normal <sup>b</sup> (n=11)		Esophagitis (n=12)		BE (n=10)	
Relative abundance (%)	75.9		50.5		54.1	
<i>P</i> value	0.043					
Follow-up tests <sup>c</sup>						
Groups compared	Phenotype					
	Normal	Esophagitis	Normal	BE	Esophagitis	BE
Relative abundance (%)	75.9	50.5	75.9		50.5	54.1
<i>P</i> value	0.016*		0.029*		0.773	

<sup>a</sup> The Omnibus test was performed using one-way ANOVA.  
<sup>b</sup> An outlier (E15) in the normal group was not included in comparisons between esophageal phenotypic groups.  
<sup>c</sup> The follow-up tests were performed with two-tailed independent *t*-test. Tests that are statistically different at the false discovery rate < 5% are marked by \*.

Table 3. Comparisons of histological phenotypes in relative abundance of *Streptococcus*

In the disease-associated type II microbiome, the decrease in the relative abundance of *Streptococcus* is compensated by an increase in the relative abundance of 24 other genera. Specifically, the most prominent increase involves *Veillonella*, *Prevotella*, *Haemophilus*, *Neisseria*, *Rothia*, *Granulicatella*, *Campylobacter*, *Porphyromonas*, *Fusobacterium*, and *Actinomyces*, many of which are Gram-negative anaerobes or microaerophiles and are putative pathogens for periodontal disease. Anaerobic (type I: 11.0% vs. type II: 38.2%,  $P = 1.2 \times 10^{-5}$ ) and microaerophilic bacteria (5.4% vs. 23.0%,  $p = 1.1 \times 10^{-4}$ ) are more abundant in the type II microbiome than in the type I microbiome (Figure 4A). Gram-negative bacteria comprise 53.4% of type II microbiome but only 14.9% of type I microbiome ( $p = 8.0 \times 10^{-10}$ ) (Figure 4B). Overall, the type II microbiome is significantly more diverse (Shannon-Wiener diversity index mean of 2.69 vs. 1.51,  $P = 1.3 \times 10^{-7}$ ) and more even (Shannon-Wiener evenness index mean 0.78 vs. 0.51,  $P = 4.2 \times 10^{-8}$ ) than the type I microbiome (Figure 5).

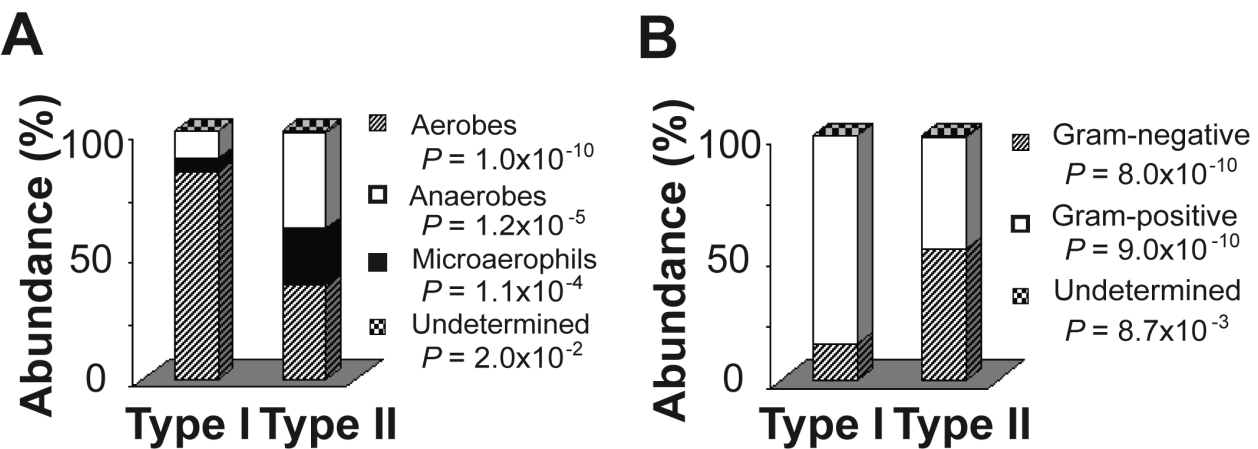


Fig. 4. Taxonomic characterization of microbiome by population of main bacterial groups. Comparisons of microbiome types according to culture conditions (Panel A) and staining properties (Panel B).

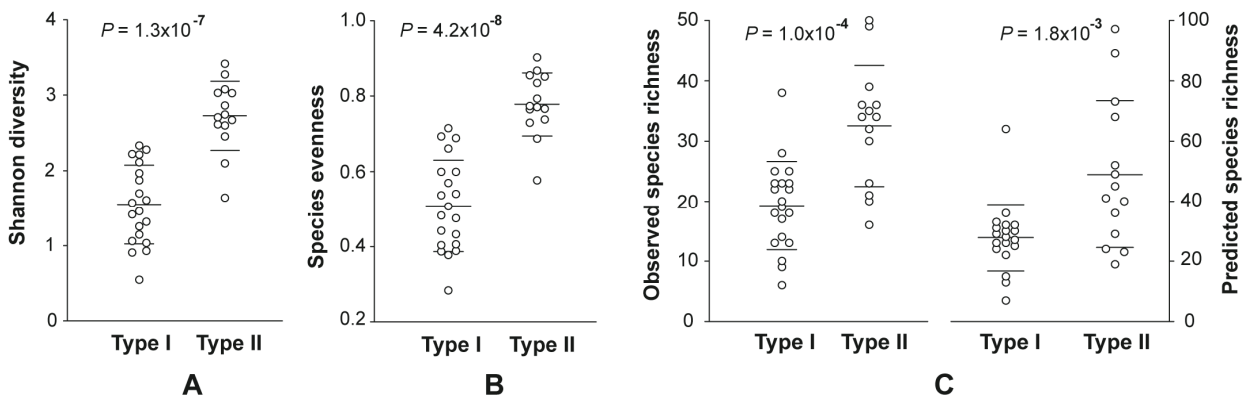


Fig. 5. Difference between the 2 types of microbiome in biologic diversity. (A) Shannon-Wiener diversity index. (B) Shannon-Wiener evenness index. (C) Richness by observed and estimated SLOtUs (Chao, 1984). Mean  $\pm$  1.96 SD is indicated by horizontal lines.

The type II microbiome appears to be the strongest ( $OR > 15$ ) amongst all known environmental factors that are associated with the pathological changes related to gastroesophageal reflux (Table 4). This finding has opened a new approach to

Category	Subcategory	Predictive factor	Predicted outcome (defining method)	Sample size	Odds ratio	95% C.I.	Reference
Host	Genetic	Immediate relatives	Heartburn (questionnaire)	1,524	2.6	1.8-3.7	Locke, 1999
		Parental family history		3,920	1.5	1.2-1.7	Mohammed, 2005
	Aging	Increasing age	GERD (ICD-9 code)	163,085	1.1	1.0-1.1	Kotzan, 2001
	Structural	Hiatus hernia			4.2	2.8-6.3	
			BE (histology)	457	3.9	2.5-6.0	Conio, 2001
			Esophagitis (endoscopy)	451	2.4	1.5-4.0	
		Papillae elongation	Heartburn (medical record)	1,128	2.2	1.5-3.2	Voutilainen, 2000
	Symptomatic	Heartburn/regurgitation	Esophagitis (endoscopy)	451	9.4	6.1-14.4	Ruigomez, 2004
			BE (histology)	457	5.8	4.0-8.4	
	Comorbid	Gallbladder disease	GERD (ICD-8 codes)	7,451	3.7	2.1-6.7	Ruigomez, 2004
		Asthma	GERD (ICD-9 code)	163,085	3.2	2.6-4.0	Kotzan, 2001
		Angina	GERD (ICD-8 codes)	7,451	3.2	2.1-4.9	Ruigomez, 2004
		Obesity	GERD (ICD-9 code)	163,085	2.8	2.1-3.6	Kotzan, 2001
		Peptic ulcer disease	GERD (ICD-8 codes)	7,451	2.5	1.7-3.6	Ruigomez, 2004
		Chest pain			2.3	1.8-2.8	
		Cough			1.7	1.4-2.1	
		Irritable bowel syndrome			1.6	1.2-2.1	
Environment	Behavioral	Tobacco	GERD (ICD-9 code)	163,085	2.6	1.9-3.5	Kotzan, 2001
		Alcohol			1.8	1.4-2.4	
	NSAID	1.8	1.6-2.1				
	Medical	Anticholinergic drug	Heartburn (questionnaire)	3,920	1.5	1.1-2.1	Mohammed, 2005
		Nitrates	GERD (ICD-8 codes)	7,451	1.5	1.1-2.0	Ruigomez, 2004
		Oral steroids			1.3	1.1-1.6	
	Bacterial	Type II microbiome	Esophagitis (histology)	24	15.4	1.5-161.0	Yang, 2009
			BE (histology)	22	16.5	1.5-183.1	

NSAID: Nonsteroidal anti-inflammatory drug.  
GERD: gastroesophageal reflux disease.  
BE: Barrett’s esophagus.  
ICD: international classification of diseases.  
ICD-8 codes for GERD: gastroesophageal reflux, esophagitis, esophageal inflammation, or heartburn.  
ICD-9 code for GERD: not specified in detail.

Table 4. Comparison of the type II microbiome with known risk factors in gastroesophageal reflux disorders



understanding the recent surge in the incidence/prevalence of GERD, BE and EAC, and suggest the possible role of dysbiosis in their pathogenesis. The diverse type II community with its larger content of Gram-negative bacteria might engage innate immune functions of the epithelial cells in a different way than the type I microbiome, owing to the release of a larger spectrum of microbial components, such as lipopolysaccharide (LPS) of Gram-negative bacteria stimulating pattern receptors (eg, Toll-like receptors). Furthermore, the type II microbiome that contains significant numbers of potential pathogens, such as *Campylobacter*, *Veillonella*, *Prevotella*, *Haemophilus*, *Neisseria*, *Porphyromonas*, *Fusobacterium*, and *Actinomyces* and a significantly higher percentage of Gram-negative bacteria, might play a role with relevance in the maintenance of inflammation. On the other hand, the type II microbiome might be secondary to changes caused by gastric reflux. The type I microbiome could represent a direct extension of the normal oral microbiome via saliva while the type II microbiome could represent regurgitated bacteria in gastric juice or a microbiome modified gastric acid by selecting against acid-sensitive bacteria in the esophagus. However, at this stage, it is unclear whether the presence of type II microbiome (or the absence of type I bacteria) plays a causal role in the pathogenesis of esophageal inflammation or BE. These hypotheses will have to be addressed by future studies, which should be conducted with a prospective design and involve a finer characterization of the microbiomes (Suerbaum, 2009). The microbiome alteration from type I to type II might prove to be an important step in the pathogenesis of esophageal tumorigenesis, and represent a biologically more plausible microbial component in GERD-BE-EAC progression. Consequently, it is essential to assess the type II microbiome and/or numbers of its potential pathogens as either a sole or a panel of biomarkers in order to decipher its relevance in GERD-BE-EAC progression.

## 6. Prognostic value of biomarkers

As discussed, biomarkers have an important role during the transition of BE to EAC. However, the diagnosis of dysplasia is still the principal marker that is monitored by endoscopic surveillance biopsies, with the aim of intervening prior to invasive adenocarcinoma. The shortcomings of this algorithm include undiagnosed disease, an unproven reduction in population mortality, and unnecessary surveillance. In addition, the current staging of EAC is based exclusively on the anatomical extent of the disease, and the tumor depth (T), lymph nodes involved (N), and the presence or absence of metastases (M); the TNM system. Despite the great number of relevant biomarkers that have been described, none are yet incorporated in a clear prognostic model in EAC. Much investigation is currently underway to identify prognostic biomarkers that may determine the best therapeutic course in this disease.

The development of cancer is generally accepted as being categorized by essential alterations in cell physiology that, when combined, dictate neoplasia: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2000). For EAC, each of these physiologic changes is associated with relevant biomarkers that have been examined for prognostic potential. Two recent reviews detail the prognostic evidence of biomarkers by using these categories of mechanisms (Lagarde et al., 2007; Ong et al., 2010).

Several biomarkers are known to enable self-sufficiency in growth signals, and could have a vital impact on the different phenotypes of EAC. In a study of 124 EAC specimens, two of three specific genotypes of cyclin D1 were found to be predictive of overall survival time (Izzo et al., 2007). In addition, several studies have looked at the prognostic relevance of EGF and TGF- $\alpha$ , two growth factors that bind to the EGFR. TGF- $\alpha$  protein expression has been shown to be significantly associated with tumor progression and lymph node metastasis (D'Errico et al., 2000). Alternatively, in patients with node-negative esophageal cancer, low-level expression of TGF- $\alpha$  is associated with a worse prognosis (Aloia et al., 2001). These apparently conflicting findings are further complicated by studies of EGFR expression in EAC specimens, as one study showed a trend in its multivariate analysis towards expression and decreased survival, (Wang et al., 2007) and another showed significantly poorer survival in association with decreased expression in a univariate analysis (Langer et al., 2006). The mechanisms behind these seemingly discordant findings of the expression of EGFR and its related growth factors are yet to be elucidated. Furthermore, an oncogene HER-2/neu related to EGFR has been shown to significantly correlate with poorer survival in EAC, and may have independent prognostic significance (Brien et al., 2000).

Other biomarkers are thought to act in progression of EAC by insensitivity to growth-inhibitory signals, including TGF- $\beta$  and APC. For TGF- $\beta$ , there is some correlation on univariate analysis that overexpression of the gene is associated with depth of tumor infiltration, nodal involvement, and lymphatic vessel invasion, in addition to having a significant negative impact on survival (von Rahden et al., 2006). This evidence was reinforced in an intraoperative study of the mean levels of TGF- $\beta$ 1 from the azygos vein in patients with esophageal cancer, as higher levels significantly correlated with poorer survival. However, the majority of these cancers were esophageal squamous cell carcinoma, as opposed to adenocarcinoma (Fukuchi et al., 2004). The APC gene, a biomarker well known for its relationship with colon adenocarcinoma, has also been studied in esophageal carcinomas. One study found a dramatic and significant increase in the methylation of APC DNA in the plasma of patients with EAC, and this was associated with reduced patient survival (Kawakami et al., 2000).

Other potential predictive biomarkers in EAC are those that affect cell cycle arrest and the evasion of apoptosis. Immunohistochemical expression of both P21 and P53 have been studied in patients with EAC before and after treatment with chemotherapy. Some data suggests that loss of P53, surprisingly, and gain in expression of P21 have both correlated with better response to chemotherapy and survival (Heeren et al., 2004). The Bcl-2 family of genes have been well studied as apoptotic regulators of programmed cell death in several malignancies. In EAC, data show that expression of Bcl-2 is ultimately associated with improved survival, but does not predict response before neoadjuvant chemotherapy, suggesting a reliance on other factors in guiding response to this therapy (Raouf et al., 2003). Furthermore, NF- $\kappa$ B, presumably by involvement in apoptosis, has been shown in a study of esophageal carcinoma specimens, with the vast majority being adenocarcinoma, to be activated and then significantly associated with overall and disease free survival after chemotherapy (Izzo et al., 2006).

The expression of COX-2, a marker of induction of apoptosis as well as increased angiogenesis, has shown promise in predicting prognosis in EAC. An original study of the intensity of immunohistochemical staining for COX-2 in EAC specimens revealed a

significant association of higher COX-2 expression and amount of distant metastases, local recurrences, and decreased survival (Buskens et al., 2002). Poorer survival with increased COX-2 expression was substantiated in a smaller study of specimens from patients who underwent esophagectomy and either died within one year, or survived three years (France et al., 2004). Finally, a later study of 100 surgically resected specimens of EAC showed significant correlation of COX-2 expression and higher T stage, N stage, risk of tumor recurrence, and overall decreased survival (Bhandari et al., 2006).

Another mechanism thought necessary for neoplastic transformation is the acquisition of limitless replicative potential, as tumors stabilize their telomeres and halt the limited number of pre-defined cell divisions before growth arrest. In a study of 46 patients with resection of Barrett's related esophageal adenocarcinoma, telomere length was studied by Southern blot analysis. The telomere-length ratio of neoplastic to normal tissue was correlated with overall survival, as patients with higher ratios had significantly poorer survival (Gertler et al., 2008).

Angiogenesis is another necessary capability of malignant tumors, and its development has also been studied in regards to prognosis of EAC. CD105, or endoglin, as well as VEGF are both known factors that perform a major role in angiogenesis. In a study of 75 patients and specimens from esophagectomies to treat adenocarcinoma, endoglin or VEGF staining of microvessels was used to account for angiogenesis. Both endoglin and VEGF showed a prognostic significance and positive correlation with angiolymphatic invasion, lymph node metastases, and poorer survival (Saad et al., 2005).

The last classic mechanism thought to be vital for neoplastic transformation is tissue invasion and metastasis. Potential prognostic biomarkers involved in this invasion in EAC include the cell adhesion molecule E-cadherin, as well as uPA and TIMP-3, proteins involved in breaking down or preserving the extracellular matrix, respectively. Reduced levels of E-cadherin, by immunohistochemical staining of 59 Barrett's related esophageal adenocarcinoma specimens, was associated with significantly poorer prognosis (Falkenback et al., 2008). By also contributing to invasiveness of tumor cells, higher uPA expression was shown to be correlated with poorer survival, and correspondingly, in another study loss of TIMP-3 correlated with poorer survival and higher disease stage (Darnton et al., 2005; Nekarda et al., 1998).

Several of the aforementioned biomarkers are promising in deciphering prognosis in EAC, and show an association with tumor invasiveness or patient survival. However, the evidence for each individual marker has typically not been well replicated, and molecularly it is unlikely that any of these markers can correctly predict survival on their own, as many of the alterations are related. More recently, microarray technology has allowed for molecular signatures that examine several targets simultaneously. This is a rapidly evolving area of research, and several studies have already examined molecular signatures in EAC in order to attempt to accurately prognosticate.

The studies using microarray technology to generate molecular prognostic signatures for EAC have also attempted to externally validate their findings. One study on 75 resected esophageal specimens generated a set of four genes found to be prognostic at the protein level, which allows for more clinical applicability. This molecular signature was found to be significantly predictive of survival based on the amount of genes dysregulated, and was externally validated in a cohort of 371 cases (Peters et al., 2010). Other studies have not

shown such promising results, as a study attempting to generate a prognostic gene expression profile for lymphatic dissemination in EAC was unsuccessful, but did identify an importance of argininosuccinate synthetase (ASS) in the development of this lymphatic dissemination (Lagarde et al., 2008). Microarray technology has also shown its potential for unexpected, though potentially important, results. In looking at response to neoadjuvant chemotherapy in EAC with microarray technology, one study showed a total of 86 genes that were differentially regulated, with the strongest difference in gene expression found with the gene encoding the ephrin B3 receptor, a tyrosine kinase receptor primarily described in the nervous system (Schauer et al., 2010).

In addition to the above methods of microarray technology, other approaches have been used to develop molecular signatures important in the prognosis of EAC. One study used protein expression profiling on 34 patients who underwent surgical resection for locally advanced EAC, and found a significant association between decreased expression of a particular protein, heat shock protein 27 (HSP27), and nonresponse to neoadjuvant chemotherapy. As overexpression of HSP27 has been shown to be associated with resistance to chemotherapy in several other cancers, including esophageal squamous cell carcinoma, this is a novel finding that needs to be further studied (Langer et al., 2008). Another study looked at a panel of genetic polymorphisms on survival outcomes in 210 esophageal carcinoma patients treated with fluorouracil, with 83% having adenocarcinoma. The study found five polymorphisms in three genes significantly associated with decreased recurrences and related disease free survival (Wu et al., 2006). Yet another study analyzed microRNA expression in 100 EAC patients, and found that reduced levels of microRNA-375 was associated with worse survival, suggesting a need for further study of microRNAs in the prognosis of this disease (Mathe et al., 2009). Finally, in a novel study looking at the total number of chromosomal aberrations in specimens of EAC using a multiplex ligation-dependent probe amplification technique, a significant negative correlation between patient survival and total number of aberrations was found. This finding may provide a more general, but still important, indicator of disease outcome (Pasello et al., 2009).

## 7. Conclusion

Overall, a lot of interest has been given to the study of biomarkers in EAC development, and to both identify prognostic indicators as well as guide therapy. Despite the large number of researched biomarkers showing a correlation with survival in EAC, it is unlikely that a single biomarker will adequately predict prognosis and survival in this disease. The wealth of data using traditional methods, in conjunction with new technologies geared towards molecular signatures, have identified both individual and panels of biomarkers important in predicting neoplastic transformation. However large-scale prospective cohorts are still needed to validate these biomarkers or molecular/microbiome signatures in development. Ultimately, these prognostic biomarkers will hopefully be incorporated into a model that can help with clinical management of patients with EAC.

## 8. Acknowledgements

Supported by grants from the National Cancer Institute and the National Institute for Allergy and Infectious Diseases K23CA107123, UH3CA140233, R01CA159036, R01AI063477, U19DE018385, as well as the RWJ Amos Medical Faculty Development Program.



## 9. References

- Abbas, T., and Dutta, A. (2009). p21 in cancer: intricate networks and multiple activities. *Nat Rev Cancer* 9, 400-414.
- Aloia, T.A., Harpole, D.H., Jr., Reed, C.E., Allegra, C., Moore, M.B., Herndon, J.E., 2nd, and D'Amico, T.A. (2001). Tumor marker expression is predictive of survival in patients with esophageal cancer. *Ann Thorac Surg* 72, 859-866.
- Arber, N., Lightdale, C., Rotterdam, H., Han, K.H., Sgambato, A., Yap, E., Ahsan, H., Finegold, J., Stevens, P.D., Green, P.H., *et al.* (1996). Increased expression of the cyclin D1 gene in Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 5, 457-459.
- Azuma, T., Suto, H., Ito, Y., Muramatsu, A., Ohtani, M., Dojo, M., Yamazaki, Y., Kuriyama, M., and Kato, T. (2002). Eradication of *Helicobacter pylori* infection induces an increase in body mass index. *Aliment Pharmacol Ther* 16 Suppl 2, 240-244.
- Baatar, D., Jones, M.K., Pai, R., Kawanaka, H., Szabo, I.L., Moon, W.S., Kitano, S., and Tarnawski, A.S. (2002). Selective cyclooxygenase-2 blocker delays healing of esophageal ulcers in rats and inhibits ulceration-triggered c-Met/hepatocyte growth factor receptor induction and extracellular signal-regulated kinase 2 activation. *Am J Pathol* 160, 963-972.
- Balish, E., and Warner, T. (2002). *Enterococcus faecalis* induces inflammatory bowel disease in interleukin-10 knockout mice. *Am J Pathol* 160, 2253-2257.
- Bani-Hani, K., Martin, I.G., Hardie, L.J., Mapstone, N., Briggs, J.A., Forman, D., and Wild, C.P. (2000). Prospective study of cyclin D1 overexpression in Barrett's esophagus: association with increased risk of adenocarcinoma. *J Natl Cancer Inst* 92, 1316-1321.
- Barthold, S.W., and Jonas, A.M. (1977). Morphogenesis of early 1, 2-dimethylhydrazine-induced lesions and latent period reduction of colon carcinogenesis in mice by a variant of *Citrobacter freundii*. *Cancer Res* 37, 4352-4360.
- Beales, I.L., and Ogunwobi, O.O. (2007). Leptin synergistically enhances the anti-apoptotic and growth-promoting effects of acid in OE33 oesophageal adenocarcinoma cells in culture. *Mol Cell Endocrinol* 274, 60-68.
- Bechi, P., Balzi, M., Becciolini, A., Maugeri, A., Raggi, C.C., Amorosi, A., and Dei, R. (1996). *Helicobacter pylori* and cell proliferation of the gastric mucosa: possible implications for gastric carcinogenesis. *Am J Gastroenterol* 91, 271-276.
- Beebe, J.L., and Koneman, E.W. (1995). Recovery of uncommon bacteria from blood: association with neoplastic disease. *Clin Microbiol Rev* 8, 336-356.
- Bhandari, P., Bateman, A.C., Mehta, R.L., Stacey, B.S., Johnson, P., Cree, I.A., Di Nicolantonio, F., and Patel, P. (2006). Prognostic significance of cyclooxygenase-2 (COX-2) expression in patients with surgically resectable adenocarcinoma of the oesophagus. *BMC Cancer* 6, 134.
- Bian, Y.S., Osterheld, M.C., Bosman, F.T., Fontollet, C., and Benhattar, J. (2000). Nuclear accumulation of beta-catenin is a common and early event during neoplastic progression of Barrett esophagus. *Am J Clin Pathol* 114, 583-590.
- Bian, Y.S., Osterheld, M.C., Fontollet, C., Bosman, F.T., and Benhattar, J. (2002). p16 inactivation by methylation of the CDKN2A promoter occurs early during neoplastic progression in Barrett's esophagus. *Gastroenterology* 122, 1113-1121.



- Blaser, M.J., Perez-Perez, G.I., Kleanthous, H., Cover, T.L., Peek, R.M., Chyou, P.H., Stemmermann, G.N., and Nomura, A. (1995). Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 55, 2111-2115.
- Bourboulia, D., and Stetler-Stevenson, W.G. (2010). Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. *Semin Cancer Biol* 20, 161-168.
- Bray, G.A. (1992). Obesity increases risk for diabetes. *Int J Obes Relat Metab Disord* 16 Suppl 4, S13-17.
- Brien, T.P., Odze, R.D., Sheehan, C.E., McKenna, B.J., and Ross, J.S. (2000). HER-2/neu gene amplification by FISH predicts poor survival in Barrett's esophagus-associated adenocarcinoma. *Hum Pathol* 31, 35-39.
- Brown, L.M., Swanson, C.A., Gridley, G., Swanson, G.M., Schoenberg, J.B., Greenberg, R.S., Silverman, D.T., Pottern, L.M., Hayes, R.B., Schwartz, A.G., *et al.* (1995). Adenocarcinoma of the esophagus: role of obesity and diet. *J Natl Cancer Inst* 87, 104-109.
- Buckley, M.F., Sweeney, K.J., Hamilton, J.A., Sini, R.L., Manning, D.L., Nicholson, R.I., deFazio, A., Watts, C.K., Musgrove, E.A., and Sutherland, R.L. (1993). Expression and amplification of cyclin genes in human breast cancer. *Oncogene* 8, 2127-2133.
- Burnat, G., Rau, T., Elshimi, E., Hahn, E.G., and Konturek, P.C. (2007). Bile acids induce overexpression of homeobox gene CDX-2 and vascular endothelial growth factor (VEGF) in human Barrett's esophageal mucosa and adenocarcinoma cell line. *Scand J Gastroenterol* 42, 1460-1465.
- Buskens, C.J., Van Rees, B.P., Sivula, A., Reitsma, J.B., Haglund, C., Bosma, P.J., Offerhaus, G.J., Van Lanschot, J.J., and Ristimäki, A. (2002). Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. *Gastroenterology* 122, 1800-1807.
- Buttar, N.S., and Wang, K.K. (2004). Mechanisms of disease: Carcinogenesis in Barrett's esophagus. *Nat Clin Pract Gastroenterol Hepatol* 1, 106-112.
- Buttar, N.S., Wang, K.K., Leontovich, O., Westcott, J.Y., Pacifico, R.J., Anderson, M.A., Krishnadath, K.K., Lutzke, L.S., and Burgart, L.J. (2002). Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. *Gastroenterology* 122, 1101-1112.
- Chao, A. (1984). Nonparametric-Estimation of the Number of Classes in a Population. *Scand J Stat* 11, 265-270.
- Cheng, M., Sexl, V., Sherr, C.J., and Roussel, M.F. (1998). Assembly of cyclin D-dependent kinase and titration of p27Kip1 regulated by mitogen-activated protein kinase kinase (MEK1). *Proc Natl Acad Sci U S A* 95, 1091-1096.
- Cheong, E., Igali, L., Harvey, I., Mole, M., Lund, E., Johnson, I.T., and Rhodes, M. (2003). Cyclo-oxygenase-2 expression in Barrett's oesophageal carcinogenesis: an immunohistochemical study. *Aliment Pharmacol Ther* 17, 379-386.
- Chirco, R., Liu, X.W., Jung, K.K., and Kim, H.R. (2006). Novel functions of TIMPs in cell signaling. *Cancer Metastasis Rev* 25, 99-113.
- Chow, W.H., Blot, W.J., Vaughan, T.L., Risch, H.A., Gammon, M.D., Stanford, J.L., Dubrow, R., Schoenberg, J.B., Mayne, S.T., Farrow, D.C., *et al.* (1998). Body mass index and

- risk of adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 90, 150-155.
- Chu, F.F., Esworthy, R.S., Chu, P.G., Longmate, J.A., Huycke, M.M., Wilczynski, S., and Doroshow, J.H. (2004). Bacteria-induced intestinal cancer in mice with disrupted Gpx1 and Gpx2 genes. *Cancer Res* 64, 962-968.
- Clement, G., Braunschweig, R., Pasquier, N., Bosman, F.T., and Benhattar, J. (2006a). Alterations of the Wnt signaling pathway during the neoplastic progression of Barrett's esophagus. *Oncogene* 25, 3084-3092.
- Clement, G., Braunschweig, R., Pasquier, N., Bosman, F.T., and Benhattar, J. (2006b). Methylation of APC, TIMP3, and TERT: a new predictive marker to distinguish Barrett's oesophagus patients at risk for malignant transformation. *J Pathol* 208, 100-107.
- Conio, M., Filiberti, R., Bianchi, S., Ferraris, R., Marchi, S., Ravelli, P., Lapertosa, G., Iaquinto, G., Sablich, R., and Giacosa, A., et al. (2001). Risk factors for Barrett's esophagus: a case-control study. *Int J Cancer* 97, 225-229.
- Cook, M.B., Greenwood, D.C., Hardie, L.J., Wild, C.P., and Forman, D. (2008). A systematic review and meta-analysis of the risk of increasing adiposity on Barrett's esophagus. *Am J Gastroenterol* 103, 292-300.
- Corley, D.A., Kubo, A., Levin, T.R., Block, G., Habel, L., Zhao, W., Leighton, P., Quesenberry, C., Rumore, G.J., and Buffler, P.A. (2007). Abdominal obesity and body mass index as risk factors for Barrett's esophagus. *Gastroenterology* 133, 34-41; quiz 311.
- Couvelard, A., Paraf, F., Gratio, V., Scoazec, J.Y., Henin, D., Degott, C., and Flejou, J.F. (2000). Angiogenesis in the neoplastic sequence of Barrett's oesophagus. Correlation with VEGF expression. *J Pathol* 192, 14-18.
- Covacci, A., Censini, S., Bugnoli, M., Petracca, R., Burrone, D., Macchia, G., Massone, A., Papini, E., Xiang, Z., Figura, N., et al. (1993). Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci U S A* 90, 5791-5795.
- Cronin, J., McAdam, E., Danikas, A., Tselepis, C., Griffiths, P., Baxter, J., Thomas, L., Manson, J., and Jenkins, G. (2011). Epidermal growth factor receptor (EGFR) is overexpressed in high-grade dysplasia and adenocarcinoma of the esophagus and may represent a biomarker of histological progression in Barrett's esophagus (BE). *Am J Gastroenterol* 106, 46-56.
- Cruz-Munoz, W., and Khokha, R. (2008). The role of tissue inhibitors of metalloproteinases in tumorigenesis and metastasis. *Crit Rev Clin Lab Sci* 45, 291-338.
- Cunningham-Rundles, S., Ahrn, S., Abuav-Nussbaum, R., and Dnistrian, A. (2002). Development of immunocompetence: role of micronutrients and microorganisms. *Nutr Rev* 60, S68-72.
- Curvers, W.L., ten Kate, F.J., Krishnadath, K.K., Visser, M., Elzer, B., Baak, L.C., Bohmer, C., Mallant-Hent, R.C., van Oijen, A., Naber, A.H., et al. (2010). Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. *Am J Gastroenterol* 105, 1523-1530.
- D'Errico, A., Barozzi, C., Fiorentino, M., Carella, R., Di Simone, M., Ferruzzi, L., Mattioli, S., and Grigioni, W.F. (2000). Role and new perspectives of transforming growth

- factor-alpha (TGF-alpha) in adenocarcinoma of the gastro-oesophageal junction. *Br J Cancer* 82, 865-870.
- Darnton, S.J., Hardie, L.J., Muc, R.S., Wild, C.P., and Casson, A.G. (2005). Tissue inhibitor of metalloproteinase-3 (TIMP-3) gene is methylated in the development of esophageal adenocarcinoma: loss of expression correlates with poor prognosis. *Int J Cancer* 115, 351-358.
- de Martel, C., Haggerty, T.D., Corley, D.A., Vogelmann, J.H., Orentreich, N., and Parsonnet, J. (2007). Serum ghrelin levels and risk of subsequent adenocarcinoma of the esophagus. *Am J Gastroenterol* 102, 1166-1172.
- Diehl, J.A., and Sherr, C.J. (1997). A dominant-negative cyclin D1 mutant prevents nuclear import of cyclin-dependent kinase 4 (CDK4) and its phosphorylation by CDK-activating kinase. *Mol Cell Biol* 17, 7362-7374.
- Dvoyrin, V.V., Sorokina, I.T., Mashinsky, V.M., Ischakova, L.D., Dianov, E.M., Kalashnikov, V.L., Yashkov, M.V., Khopin, V.F., and Guryanov, A.N. (2011). Tm<sup>3+</sup>-doped CW fiber laser based on a highly GeO<sub>2</sub>-doped dispersion-shifted fiber. *Opt Express* 19, 7992-7999.
- Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., and Relman, D.A. (2005). Diversity of the human intestinal microbial flora. *Science* 308, 1635-1638.
- Edelstein, Z.R., Farrow, D.C., Bronner, M.P., Rosen, S.N., and Vaughan, T.L. (2007). Central adiposity and risk of Barrett's esophagus. *Gastroenterology* 133, 403-411.
- Eisen, G.M., Sandler, R.S., Murray, S., and Gottfried, M. (1997). The relationship between gastroesophageal reflux disease and its complications with Barrett's esophagus. *Am J Gastroenterol* 92, 27-31.
- El-Omar, E.M., Rabkin, C.S., Gammon, M.D., Vaughan, T.L., Risch, H.A., Schoenberg, J.B., Stanford, J.L., Mayne, S.T., Goedert, J., Blot, W.J., *et al.* (2003). Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 124, 1193-1201.
- El-Serag, H. (2008). Role of obesity in GORD-related disorders. *Gut* 57, 281-284.
- El-Serag, H.B., Ergun, G.A., Pandolfino, J., Fitzgerald, S., Tran, T., and Kramer, J.R. (2007). Obesity increases oesophageal acid exposure. *Gut* 56, 749-755.
- El-Serag, H.B., Graham, D.Y., Satia, J.A., and Rabeneck, L. (2005). Obesity is an independent risk factor for GERD symptoms and erosive esophagitis. *Am J Gastroenterol* 100, 1243-1250.
- El-Serag, H.B., Tran, T., Richardson, P., and Ergun, G. (2006). Anthropometric correlates of intragastric pressure. *Scand J Gastroenterol* 41, 887-891.
- Eloubeidi, M.A., and Provenzale, D. (2001). Clinical and demographic predictors of Barrett's esophagus among patients with gastroesophageal reflux disease: a multivariable analysis in veterans. *J Clin Gastroenterol* 33, 306-309.
- Engle, S.J., Ormsby, I., Pawlowski, S., Boivin, G.P., Croft, J., Balish, E., and Doetschman, T. (2002). Elimination of colon cancer in germ-free transforming growth factor beta 1-deficient mice. *Cancer Res* 62, 6362-6366.
- Erdman, S.E., Poutahidis, T., Tomczak, M., Rogers, A.B., Cormier, K., Plank, B., Horwitz, B.H., and Fox, J.G. (2003). CD4<sup>+</sup> CD25<sup>+</sup> regulatory T lymphocytes inhibit

- microbially induced colon cancer in Rag2-deficient mice. *Am J Pathol* 162, 691-702.
- Evan, G.I., and Vousden, K.H. (2001). Proliferation, cell cycle and apoptosis in cancer. *Nature* 411, 342-348.
- Falkenback, D., Nilbert, M., Oberg, S., and Johansson, J. (2008). Prognostic value of cell adhesion in esophageal adenocarcinomas. *Dis Esophagus* 21, 97-102.
- Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C., and Parkin, D.M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 127, 2893-2917.
- Figueiredo, C., Machado, J.C., Pharoah, P., Seruca, R., Sousa, S., Carvalho, R., Capelinha, A.F., Quint, W., Caldas, C., van Doorn, L.J., *et al.* (2002). *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. *J Natl Cancer Inst* 94, 1680-1687.
- Fisher, B.L., Pennathur, A., Mutnick, J.L., and Little, A.G. (1999). Obesity correlates with gastroesophageal reflux. *Dig Dis Sci* 44, 2290-2294.
- Flegal, K.M., Carroll, M.D., Ogden, C.L., and Curtin, L.R. (2010). Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* 303, 235-241.
- France, M., Drew, P.A., Dodd, T., and Watson, D.I. (2004). Cyclo-oxygenase-2 expression in esophageal adenocarcinoma as a determinant of clinical outcome following esophagectomy. *Dis Esophagus* 17, 136-140.
- Francois, F., Roper, J., Goodman, A.J., Pei, Z., Ghumman, M., Mourad, M., de Perez, A.Z., Perez-Perez, G.I., Tseng, C.H., and Blaser, M.J. (2008). The association of gastric leptin with oesophageal inflammation and metaplasia. *Gut* 57, 16-24.
- Fu, M., Rao, M., Bouras, T., Wang, C., Wu, K., Zhang, X., Li, Z., Yao, T.P., and Pestell, R.G. (2005). Cyclin D1 inhibits peroxisome proliferator-activated receptor gamma-mediated adipogenesis through histone deacetylase recruitment. *J Biol Chem* 280, 16934-16941.
- Fu, M., Wang, C., Li, Z., Sakamaki, T., and Pestell, R.G. (2004). Minireview: Cyclin D1: normal and abnormal functions. *Endocrinology* 145, 5439-5447.
- Fuccio, L., Eusebi, L.H., Zagari, R.M., and Bazzoli, F. (2009). *Helicobacter pylori* eradication treatment reduces but does not abolish the risk of gastric cancer. *Am J Gastroenterol* 104, 3100; author reply 3101-3102.
- Fukuchi, M., Miyazaki, T., Fukai, Y., Nakajima, M., Sohda, M., Masuda, N., Manda, R., Tsukada, K., Kato, H., and Kuwano, H. (2004). Plasma level of transforming growth factor beta1 measured from the azygos vein predicts prognosis in patients with esophageal cancer. *Clin Cancer Res* 10, 2738-2741.
- Gagliardi, D., Makihara, S., Corsi, P.R., Viana Ade, T., Wiczer, M.V., Nakakubo, S., and Mimica, L.M. (1998). Microbial flora of the normal esophagus. *Dis Esophagus* 11, 248-250.
- Gartel, A.L. (2006). Is p21 an oncogene? *Mol Cancer Ther* 5, 1385-1386.
- Gerdes, J. (1990). Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluations in human malignancies. *Semin Cancer Biol* 1, 199-206.



- Gertler, R., Doll, D., Maak, M., Feith, M., and Rosenberg, R. (2008). Telomere length and telomerase subunits as diagnostic and prognostic biomarkers in Barrett carcinoma. *Cancer* 112, 2173-2180.
- Giles, R.H., van Es, J.H., and Clevers, H. (2003). Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta* 1653, 1-24.
- Greenblatt, M.S., Bennett, W.P., Hollstein, M., and Harris, C.C. (1994). Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 54, 4855-4878.
- Hampel, H., Abraham, N.S., and El-Serag, H.B. (2005). Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. *Ann Intern Med* 143, 199-211.
- Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. *Cell* 100, 57-70.
- Hardie, L.J., Darnton, S.J., Wallis, Y.L., Chauhan, A., Hainaut, P., Wild, C.P., and Casson, A.G. (2005). p16 expression in Barrett's esophagus and esophageal adenocarcinoma: association with genetic and epigenetic alterations. *Cancer Lett* 217, 221-230.
- Heeren, P.A., Kloppenberg, F.W., Hollema, H., Mulder, N.H., Nap, R.E., and Plukker, J.T. (2004). Predictive effect of p53 and p21 alteration on chemotherapy response and survival in locally advanced adenocarcinoma of the esophagus. *Anticancer Res* 24, 2579-2583.
- Herbst, J.J., Berenson, M.M., McCloskey, D.W., and Wiser, W.C. (1978). Cell proliferation in esophageal columnar epithelium (Barrett's esophagus). *Gastroenterology* 75, 683-687.
- Hinds, P.W., Finlay, C.A., Quartin, R.S., Baker, S.J., Fearon, E.R., Vogelstein, B., and Levine, A.J. (1990). Mutant p53 DNA clones from human colon carcinomas cooperate with ras in transforming primary rat cells: a comparison of the "hot spot" mutant phenotypes. *Cell Growth Differ* 1, 571-580.
- Housa, D., Housova, J., Vernerova, Z., and Haluzik, M. (2006). Adipocytokines and cancer. *Physiol Res* 55, 233-244.
- Huang, J.Q., Zheng, G.F., Sumanac, K., Irvine, E.J., and Hunt, R.H. (2003). Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 125, 1636-1644.
- IARC (1994). Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 61, 1-241.
- Islami, F., and Kamangar, F. (2008). *Helicobacter pylori* and esophageal cancer risk: a meta-analysis. *Cancer Prev Res (Phila)* 1, 329-338.
- Izzo, J.G., Malhotra, U., Wu, T.T., Ensor, J., Luthra, R., Lee, J.H., Swisher, S.G., Liao, Z., Chao, K.S., Hittelman, W.N., *et al.* (2006). Association of activated transcription factor nuclear factor kappaB with chemoradiation resistance and poor outcome in esophageal carcinoma. *J Clin Oncol* 24, 748-754.
- Izzo, J.G., Wu, T.T., Wu, X., Ensor, J., Luthra, R., Pan, J., Correa, A., Swisher, S.G., Chao, C.K., Hittelman, W.N., *et al.* (2007). Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma. *J Clin Oncol* 25, 698-707.



- Jang, E.J., Park, S.W., Park, J.S., Park, S.J., Hahm, K.B., Paik, S.Y., Sin, M.K., Lee, E.S., Oh, S.W., Park, C.Y., *et al.* (2008). The influence of the eradication of *Helicobacter pylori* on gastric ghrelin, appetite, and body mass index in patients with peptic ulcer disease. *J Gastroenterol Hepatol* 23 Suppl 2, S278-285.
- Jiang, Y., Goldberg, I.D., and Shi, Y.E. (2002). Complex roles of tissue inhibitors of metalloproteinases in cancer. *Oncogene* 21, 2245-2252.
- Jones, A.D., Bacon, K.D., Jobe, B.A., Sheppard, B.C., Deveney, C.W., and Rutten, M.J. (2003). *Helicobacter pylori* induces apoptosis in Barrett's-derived esophageal adenocarcinoma cells. *J Gastrointest Surg* 7, 68-76.
- Kado, S., Uchida, K., Funabashi, H., Iwata, S., Nagata, Y., Ando, M., Onoue, M., Matsuoka, Y., Ohwaki, M., and Morotomi, M. (2001). Intestinal microflora are necessary for development of spontaneous adenocarcinoma of the large intestine in T-cell receptor beta chain and p53 double-knockout mice. *Cancer Res* 61, 2395-2398.
- Kaur, B.S., Khamnehei, N., Iravani, M., Namburu, S.S., Lin, O., and Triadafilopoulos, G. (2002). Rofecoxib inhibits cyclooxygenase 2 expression and activity and reduces cell proliferation in Barrett's esophagus. *Gastroenterology* 123, 60-67.
- Kawaguchi-Miyashita, M., Shimada, S., Kurosu, H., Kato-Nagaoka, N., Matsuoka, Y., Ohwaki, M., Ishikawa, H., and Nanno, M. (2001). An accessory role of TCR $\gamma$ delta (+) cells in the exacerbation of inflammatory bowel disease in TCR $\alpha$  mutant mice. *Eur J Immunol* 31, 980-988.
- Kawakami, K., Brabender, J., Lord, R.V., Groshen, S., Greenwald, B.D., Krasna, M.J., Yin, J., Fleisher, A.S., Abraham, J.M., Beer, D.G., *et al.* (2000). Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. *J Natl Cancer Inst* 92, 1805-1811.
- Kim, J.K., and Diehl, J.A. (2009). Nuclear cyclin D1: an oncogenic driver in human cancer. *J Cell Physiol* 220, 292-296.
- Kojima, M., and Kangawa, K. (2005). Ghrelin: structure and function. *Physiol Rev* 85, 495-522.
- Konturek, P.C., Burnat, G., Rau, T., Hahn, E.G., and Konturek, S. (2008). Effect of adiponectin and ghrelin on apoptosis of Barrett adenocarcinoma cell line. *Dig Dis Sci* 53, 597-605.
- Konturek, P.C., Kania, J., Burnat, G., Hahn, E.G., and Konturek, S.J. (2005). Prostaglandins as mediators of COX-2 derived carcinogenesis in gastrointestinal tract. *J Physiol Pharmacol* 56 Suppl 5, 57-73.
- Konturek, P.C., Nikiforuk, A., Kania, J., Raithel, M., Hahn, E.G., and Muhldorfer, S. (2004). Activation of NF $\kappa$ B represents the central event in the neoplastic progression associated with Barrett's esophagus: a possible link to the inflammation and overexpression of COX-2, PPAR $\gamma$  and growth factors. *Dig Dis Sci* 49, 1075-1083.
- Kornbluth, A.A., Danzig, J.B., and Bernstein, L.H. (1989). *Clostridium septicum* infection and associated malignancy. Report of 2 cases and review of the literature. *Medicine (Baltimore)* 68, 30-37.

- Kotzan, J., Wade, W., and Yu, H.H. (2001). Assessing NSAID Prescription Use as a Predisposing Factor for Gastroesophageal Reflux Disease in a Medicaid Population. *Pharm Res* 18,1367-1372.
- Krishnadath, K.K., Tilanus, H.W., van Blankenstein, M., Bosman, F.T., and Mulder, A.H. (1995). Accumulation of p53 protein in normal, dysplastic, and neoplastic Barrett's oesophagus. *J Pathol* 175, 175-180.
- Kubo, A., and Corley, D.A. (2006). Body mass index and adenocarcinomas of the esophagus or gastric cardia: a systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 15, 872-878.
- Kuipers, E.J., Perez-Perez, G.I., Meuwissen, S.G., and Blaser, M.J. (1995). *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. *J Natl Cancer Inst* 87, 1777-1780.
- Kusters, J.G., van Vliet, A.H., and Kuipers, E.J. (2006). Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev* 19, 449-490.
- LaBaer, J., Garrett, M.D., Stevenson, L.F., Slingerland, J.M., Sandhu, C., Chou, H.S., Fattaey, A., and Harlow, E. (1997). New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 11, 847-862.
- Lagarde, S.M., ten Kate, F.J., Richel, D.J., Offerhaus, G.J., and van Lanschot, J.J. (2007). Molecular prognostic factors in adenocarcinoma of the esophagus and gastroesophageal junction. *Ann Surg Oncol* 14, 977-991.
- Lagarde, S.M., Ver Loren van Themaat, P.E., Moerland, P.D., Gilhuijs-Pederson, L.A., Ten Kate, F.J., Reitsma, P.H., van Kampen, A.H., Zwinderman, A.H., Baas, F., and van Lanschot, J.J. (2008). Analysis of gene expression identifies differentially expressed genes and pathways associated with lymphatic dissemination in patients with adenocarcinoma of the esophagus. *Ann Surg Oncol* 15, 3459-3470.
- Lagergren, J., Bergstrom, R., Lindgren, A., and Nyren, O. (1999a). Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N Engl J Med* 340, 825-831.
- Lagergren, J., Bergstrom, R., and Nyren, O. (1999b). Association between body mass and adenocarcinoma of the esophagus and gastric cardia. *Ann Intern Med* 130, 883-890.
- Lambert, D.M., Marceau, S., and Forse, R.A. (2005). Intra-abdominal pressure in the morbidly obese. *Obes Surg* 15, 1225-1232.
- Langer, R., Ott, K., Specht, K., Becker, K., Lordick, F., Burian, M., Herrmann, K., Schrattenholz, A., Cahill, M.A., Schwaiger, M., *et al.* (2008). Protein expression profiling in esophageal adenocarcinoma patients indicates association of heat-shock protein 27 expression and chemotherapy response. *Clin Cancer Res* 14, 8279-8287.
- Langer, R., Von Rahden, B.H., Nahrig, J., Von Weyhern, C., Reiter, R., Feith, M., Stein, H.J., Siewert, J.R., Hofler, H., and Sarbia, M. (2006). Prognostic significance of expression patterns of c-erbB-2, p53, p16INK4A, p27KIP1, cyclin D1 and epidermal growth factor receptor in oesophageal adenocarcinoma: a tissue microarray study. *J Clin Pathol* 59, 631-634.
- Lanuti, M., Liu, G., Goodwin, J.M., Zhai, R., Fuchs, B.C., Asomaning, K., Su, L., Nishioka, N.S., Tanabe, K.K., and Christiani, D.C. (2008). A functional epidermal growth

- factor (EGF) polymorphism, EGF serum levels, and esophageal adenocarcinoma risk and outcome. *Clin Cancer Res* 14, 3216-3222.
- Laufs, S., Schumacher, J., and Allgayer, H. (2006). Urokinase-receptor (u-PAR): an essential player in multiple games of cancer: a review on its role in tumor progression, invasion, metastasis, proliferation/dormancy, clinical outcome and minimal residual disease. *Cell Cycle* 5, 1760-1771.
- Lederberg, J., and McCray, A.T. (2001). 'Ome sweet 'omics - A genealogical treasury of words. *Scientist* 15, 8-8.
- Lehrbach, D.M., Cecconello, I., Ribeiro Jr, U., Capelozzi, V.L., Ab'saber, A.M., and Alves, V.A. (2009). Adenocarcinoma of the esophagogastric junction: relationship between clinicopathological data and p53, cyclin D1 and Bcl-2 immunoexpressions. *Arq Gastroenterol* 46, 315-320.
- Levine, A.J. (1997). p53, the cellular gatekeeper for growth and division. *Cell* 88, 323-331.
- Levine, A.J., Hu, W., and Feng, Z. (2006). The P53 pathway: what questions remain to be explored? *Cell Death Differ* 13, 1027-1036.
- Li, Y., Wo, J.M., Ray, M.B., Jones, W., Su, R.R., Ellis, S., and Martin, R.C. (2006). Cyclooxygenase-2 and epithelial growth factor receptor up-regulation during progression of Barrett's esophagus to adenocarcinoma. *World J Gastroenterol* 12, 928-934.
- Lipetz, L.E. (1984). A new method for determining peak absorbance of dense pigment samples and its application to the cone oil droplets of *Emydoidea blandingii*. *Vision Res* 24, 567-604.
- Liu, Y., Yeh, N., Zhu, X.H., Leversha, M., Cordon-Cardo, C., Ghossein, R., Singh, B., Holland, E., and Koff, A. (2007). Somatic cell type specific gene transfer reveals a tumor-promoting function for p21(Waf1/Cip1). *EMBO J* 26, 4683-4693.
- Locke, G.R., 3rd, Talley, N.J., Fett, S.L., Zinsmeister, A.R., and Melton, L.J., 3rd (1999). Risk factors associated with symptoms of gastroesophageal reflux. *Am J Med* 106, 642-649.
- Logan, C.Y., and Nusse, R. (2004). The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20, 781-810.
- Louw, J.A., Kidd, M.S., Kummer, A.F., Taylor, K., Kotze, U., and Hanslo, D. (2001). The relationship between *Helicobacter pylori* infection, the virulence genotypes of the infecting strain and gastric cancer in the African setting. *Helicobacter* 6, 268-273.
- Ma, T., Van Tine, B.A., Wei, Y., Garrett, M.D., Nelson, D., Adams, P.D., Wang, J., Qin, J., Chow, L.T., and Harper, J.W. (2000). Cell cycle-regulated phosphorylation of p220(NPAT) by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. *Genes Dev* 14, 2298-2313.
- Madani, K., Zhao, R., Lim, H.J., and Casson, A.G. (2010). Prognostic value of p53 mutations in oesophageal adenocarcinoma: final results of a 15-year prospective study. *Eur J Cardiothorac Surg* 37, 1427-1432.
- Maley, C.C., Galipeau, P.C., Li, X., Sanchez, C.A., Paulson, T.G., and Reid, B.J. (2004). Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. *Cancer Res* 64, 3414-3427.

- Marone, M., Scambia, G., Giannitelli, C., Ferrandina, G., Masciullo, V., Bellacosa, A., Benedetti-Panici, P., and Mancuso, S. (1998). Analysis of cyclin E and CDK2 in ovarian cancer: gene amplification and RNA overexpression. *Int J Cancer* 75, 34-39.
- Marshall, B.J., and Warren, J.R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1, 1311-1315.
- Masuda, Y., Tanaka, T., Inomata, N., Ohnuma, N., Tanaka, S., Itoh, Z., Hosoda, H., Kojima, M., and Kangawa, K. (2000). Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* 276, 905-908.
- Mathe, E.A., Nguyen, G.H., Bowman, E.D., Zhao, Y., Budhu, A., Schetter, A.J., Braun, R., Reimers, M., Kumamoto, K., Hughes, D., *et al.* (2009). MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res* 15, 6192-6200.
- McMahon, B., and Kwaan, H.C. (2008). The plasminogen activator system and cancer. *Pathophysiol Haemost Thromb* 36, 184-194.
- Mercer, C.D., Rue, C., Hanelin, L., and Hill, L.D. (1985). Effect of obesity on esophageal transit. *Am J Surg* 149, 177-181.
- Metzger, R., Schneider, P.M., Warnecke-Eberz, U., Brabender, J., and Holscher, A.H. (2004). Molecular biology of esophageal cancer. *Onkologie* 27, 200-206.
- Miehlke, S., Yu, J., Schuppler, M., Frings, C., Kirsch, C., Negraszus, N., Morgner, A., Stolte, M., Ehninger, G., and Bayerdorffer, E. (2001). *Helicobacter pylori* vacA, iceA, and cagA status and pattern of gastritis in patients with malignant and benign gastroduodenal disease. *Am J Gastroenterol* 96, 1008-1013.
- Miller, C.T., Moy, J.R., Lin, L., Schipper, M., Normolle, D., Brenner, D.E., Iannettoni, M.D., Orringer, M.B., and Beer, D.G. (2003). Gene amplification in esophageal adenocarcinomas and Barrett's with high-grade dysplasia. *Clin Cancer Res* 9, 4819-4825.
- Mobius, C., Stein, H.J., Becker, I., Feith, M., Theisen, J., Gais, P., Jutting, U., and Siewert, J.R. (2003). The 'angiogenic switch' in the progression from Barrett's metaplasia to esophageal adenocarcinoma. *Eur J Surg Oncol* 29, 890-894.
- Mohammed, I., Cherkas, L.F., Riley, S.A., Spector, T.D., and Trudgill, N.J. (2005). Genetic influences in irritable bowel syndrome: a twin study. *Am J Gastroenterol* 100, 1340-1344.
- Murray, L., Johnston, B., Lane, A., Harvey, I., Donovan, J., Nair, P., and Harvey, R. (2003). Relationship between body mass and gastro-oesophageal reflux symptoms: The Bristol *Helicobacter* Project. *Int J Epidemiol* 32, 645-650.
- Murray, L., Sedo, A., Scott, M., McManus, D., Sloan, J.M., Hardie, L.J., Forman, D., and Wild, C.P. (2006). TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. *Gut* 55, 1390-1397.
- Narikiyo, M., Tanabe, C., Yamada, Y., Igaki, H., Tachimori, Y., Kato, H., Muto, M., Montesano, R., Sakamoto, H., Nakajima, Y., *et al.* (2004). Frequent and preferential infection of *Treponema denticola*, *Streptococcus mitis*, and *Streptococcus anginosus* in esophageal cancers. *Cancer Sci* 95, 569-574.
- Nekarda, H., Schlegel, P., Schmitt, M., Stark, M., Mueller, J.D., Fink, U., and Siewert, J.R. (1998). Strong prognostic impact of tumor-associated urokinase-type plasminogen

- activator in completely resected adenocarcinoma of the esophagus. *Clin Cancer Res* 4, 1755-1763.
- Neuman, E., Ladha, M.H., Lin, N., Upton, T.M., Miller, S.J., DiRenzo, J., Pestell, R.G., Hinds, P.W., Dowdy, S.F., Brown, M., *et al.* (1997). Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4. *Mol Cell Biol* 17, 5338-5347.
- Newman, J.V., Kosaka, T., Sheppard, B.J., Fox, J.G., and Schauer, D.B. (2001). Bacterial infection promotes colon tumorigenesis in Apc(Min/+) mice. *J Infect Dis* 184, 227-230.
- Nishida, M., Funahashi, T., and Shimomura, I. (2007). Pathophysiological significance of adiponectin. *Med Mol Morphol* 40, 55-67.
- Nwokolo, C.U., Freshwater, D.A., O'Hare, P., and Randeva, H.S. (2003). Plasma ghrelin following cure of *Helicobacter pylori*. *Gut* 52, 637-640.
- Nzeako, U.C., Guicciardi, M.E., Yoon, J.H., Bronk, S.F., and Gores, G.J. (2002). COX-2 inhibits Fas-mediated apoptosis in cholangiocarcinoma cells. *Hepatology* 35, 552-559.
- Ogunwobi, O., Mutungi, G., and Beales, I.L. (2006). Leptin stimulates proliferation and inhibits apoptosis in Barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-dependent, prostaglandin-E2-mediated transactivation of the epidermal growth factor receptor and c-Jun NH2-terminal kinase activation. *Endocrinology* 147, 4505-4516.
- Ogunwobi, O.O., and Beales, I.L. (2008a). Globular adiponectin, acting via adiponectin receptor-1, inhibits leptin-stimulated oesophageal adenocarcinoma cell proliferation. *Mol Cell Endocrinol* 285, 43-50.
- Ogunwobi, O.O., and Beales, I.L. (2008b). Leptin stimulates the proliferation of human oesophageal adenocarcinoma cells via HB-EGF and Tgfbeta mediated transactivation of the epidermal growth factor receptor. *Br J Biomed Sci* 65, 121-127.
- Ong, C.A., Lao-Sirieix, P., and Fitzgerald, R.C. (2010). Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: predictors of progression and prognosis. *World J Gastroenterol* 16, 5669-5681.
- Onwuegbusi, B.A., Aitchison, A., Chin, S.F., Kranjac, T., Mills, I., Huang, Y., Lao-Sirieix, P., Caldas, C., and Fitzgerald, R.C. (2006). Impaired transforming growth factor beta signalling in Barrett's carcinogenesis due to frequent SMAD4 inactivation. *Gut* 55, 764-774.
- Onwuegbusi, B.A., Rees, J.R., Lao-Sirieix, P., and Fitzgerald, R.C. (2007). Selective loss of TGFbeta Smad-dependent signalling prevents cell cycle arrest and promotes invasion in oesophageal adenocarcinoma cell lines. *PLoS One* 2, e177.
- Pandolfino, J.E., El-Serag, H.B., Zhang, Q., Shah, N., Ghosh, S.K., and Kahrilas, P.J. (2006). Obesity: a challenge to esophagogastric junction integrity. *Gastroenterology* 130, 639-649.
- Panwalker, A.P. (1988). Unusual infections associated with colorectal cancer. *Rev Infect Dis* 10, 347-364.
- Parkin, D.M., Bray, F., Ferlay, J., and Pisani, P. (2005). Global cancer statistics, 2002. *CA Cancer J Clin* 55, 74-108.



- Parsonnet, J., Friedman, G.D., Orentreich, N., and Vogelman, H. (1997). Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. *Gut* 40, 297-301.
- Pasello, G., Agata, S., Bonaldi, L., Corradin, A., Montagna, M., Zamarchi, R., Parenti, A., Cagol, M., Zaninotto, G., Ruol, A., *et al.* (2009). DNA copy number alterations correlate with survival of esophageal adenocarcinoma patients. *Mod Pathol* 22, 58-65.
- Peek, R.M., Jr., and Blaser, M.J. (2002). *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2, 28-37.
- Pei, Z., Bini, E.J., Yang, L., Zhou, M., Francois, F., and Blaser, M.J. (2004). Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci U S A* 101, 4250-4255.
- Pei, Z., Yang, L., Peek, R.M., Jr Levine, S.M., Pride, D.T., and Blaser, M.J. (2005). Bacterial biota in reflux esophagitis and Barretts esophagus. *World J Gastroenterol* 11, 7277-7283.
- Pellish, L.J., Hermos, J.A., and Eastwood, G.L. (1980). Cell proliferation in three types of Barrett's epithelium. *Gut* 21, 26-31.
- Peters, C.J., Rees, J.R., Hardwick, R.H., Hardwick, J.S., Vowler, S.L., Ong, C.A., Zhang, C., Save, V., O'Donovan, M., Rassl, D., *et al.* (2010). A 4-gene signature predicts survival of patients with resected adenocarcinoma of the esophagus, junction, and gastric cardia. *Gastroenterology* 139, 1995-2004 e1915.
- Petros, A.M., Gunasekera, A., Xu, N., Olejniczak, E.T., and Fesik, S.W. (2004). Defining the p53 DNA-binding domain/Bcl-x(L)-binding interface using NMR. *FEBS Lett* 559, 171-174.
- Polk, D.B., and Peek, R.M., Jr. (2010). *Helicobacter pylori*: gastric cancer and beyond. *Nat Rev Cancer* 10, 403-414.
- Pontioli, A.E., and Galli, L. (1998). Duration of obesity is a risk factor for non-insulin-dependent diabetes mellitus, not for arterial hypertension or for hyperlipidaemia. *Acta Diabetol* 35, 130-136.
- Pounder, R.E., and Ng, D. (1995). The prevalence of *Helicobacter pylori* infection in different countries. *Aliment Pharmacol Ther* 9 Suppl 2, 33-39.
- Powell, E.L., Leoni, L.M., Canto, M.I., Forastiere, A.A., Iocobuzio-Donahue, C.A., Wang, J.S., Maitra, A., and Montgomery, E. (2005). Concordant loss of MTAP and p16/CDKN2A expression in gastroesophageal carcinogenesis: evidence of homozygous deletion in esophageal noninvasive precursor lesions and therapeutic implications. *Am J Surg Pathol* 29, 1497-1504.
- Preston-Martin, S., Pike, M.C., Ross, R.K., Jones, P.A., and Henderson, B.E. (1990). Increased cell division as a cause of human cancer. *Cancer Res* 50, 7415-7421.
- Raghunath, A., Hungin, A.P., Wooff, D., and Childs, S. (2003). Prevalence of *Helicobacter pylori* in patients with gastro-oesophageal reflux disease: systematic review. *BMJ* 326, 737.
- Ramel, S., Reid, B.J., Sanchez, C.A., Blount, P.L., Levine, D.S., Neshat, K., Haggitt, R.C., Dean, P.J., Thor, K., and Rabinovitch, P.S. (1992). Evaluation of p53 protein expression in Barrett's esophagus by two-parameter flow cytometry. *Gastroenterology* 102, 1220-1228.

- Raouf, A.A., Evoy, D.A., Carton, E., Mulligan, E., Griffin, M.M., and Reynolds, J.V. (2003). Loss of Bcl-2 expression in Barrett's dysplasia and adenocarcinoma is associated with tumor progression and worse survival but not with response to neoadjuvant chemoradiation. *Dis Esophagus* 16, 17-23.
- Rath, H.C., Herfarth, H.H., Ikeda, J.S., Grenther, W.B., Hamm, T.E., Jr., Balish, E., Taurog, J.D., Hammer, R.E., Wilson, K.H., and Sartor, R.B. (1996). Normal luminal bacteria, especially *Bacteroides* species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. *J Clin Invest* 98, 945-953.
- Rees, J.R., Onwuegbusi, B.A., Save, V.E., Alderson, D., and Fitzgerald, R.C. (2006). In vivo and in vitro evidence for transforming growth factor-beta1-mediated epithelial to mesenchymal transition in esophageal adenocarcinoma. *Cancer Res* 66, 9583-9590.
- Reid, B.J., Sanchez, C.A., Blount, P.L., and Levine, D.S. (1993). Barrett's esophagus: cell cycle abnormalities in advancing stages of neoplastic progression. *Gastroenterology* 105, 119-129.
- Rhead, J.L., Letley, D.P., Mohammadi, M., Hussein, N., Mohagheghi, M.A., Eshagh Hosseini, M., and Atherton, J.C. (2007). A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. *Gastroenterology* 133, 926-936.
- Richter, J.E., Falk, G.W., and Vaezi, M.F. (1998). *Helicobacter pylori* and gastroesophageal reflux disease: the bug may not be all bad. *Am J Gastroenterol* 93, 1800-1802.
- Rocco, J.W., and Sidransky, D. (2001). p16(MTS-1/CDKN2/INK4a) in cancer progression. *Exp Cell Res* 264, 42-55.
- Rokkas, T., Pistiolas, D., Sechopoulos, P., Robotis, I., and Margantinis, G. (2007). Relationship between *Helicobacter pylori* infection and esophageal neoplasia: a meta-analysis. *Clin Gastroenterol Hepatol* 5, 1413-1417, 1417 e1411-1412.
- Roninson, I.B. (2002). Oncogenic functions of tumour suppressor p21(Waf1/Cip1/Sdi1): association with cell senescence and tumour-promoting activities of stromal fibroblasts. *Cancer Lett* 179, 1-14.
- Ronkainen, J., Aro, P., Storskrubb, T., Johansson, S.E., Lind, T., Bolling-Sternevald, E., Vieth, M., Stolte, M., Talley, N.J., and Agreus, L. (2005). Prevalence of Barrett's esophagus in the general population: an endoscopic study. *Gastroenterology* 129, 1825-1831.
- Roper, J., Francois, F., Shue, P.L., Mourad, M.S., Pei, Z., Olivares de Perez, A.Z., Perez-Perez, G.I., Tseng, C.H., and Blaser, M.J. (2008). Leptin and ghrelin in relation to *Helicobacter pylori* status in adult males. *J Clin Endocrinol Metab* 93, 2350-2357.
- Rubenstein, J.H., Dahlkemper, A., Kao, J.Y., Zhang, M., Morgenstern, H., McMahon, L., and Inadomi, J.M. (2008). A pilot study of the association of low plasma adiponectin and Barrett's esophagus. *Am J Gastroenterol* 103, 1358-1364.
- Rubenstein, J.H., Kao, J.Y., Madanick, R.D., Zhang, M., Wang, M., Spacek, M.B., Donovan, J.L., Bright, S.D., and Shaheen, N.J. (2009). Association of adiponectin multimers with Barrett's oesophagus. *Gut* 58, 1583-1589.
- Ruigomez, A., Rodriguez, L.A.G., Wallander, M.A., Johansson, S., Graffner, H., and Dent, J. (2004). Natural history of gastro-oesophageal reflux disease diagnosed in general practice. *Aliment Pharmacol Ther* 20, 751-760.

- Ruhl, C.E., and Everhart, J.E. (1999). Overweight, but not high dietary fat intake, increases risk of gastroesophageal reflux disease hospitalization: the NHANES I Epidemiologic Followup Study. First National Health and Nutrition Examination Survey. *Ann Epidemiol* 9, 424-435.
- Rygiel, A.M., Milano, F., Ten Kate, F.J., Schaap, A., Wang, K.K., Peppelenbosch, M.P., Bergman, J.J., and Krishnadath, K.K. (2008). Gains and amplifications of c-myc, EGFR, and 20.q13 loci in the no dysplasia-dysplasia-adenocarcinoma sequence of Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 17, 1380-1385.
- Saad, R.S., El-Gohary, Y., Memari, E., Liu, Y.L., and Silverman, J.F. (2005). Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in esophageal adenocarcinoma. *Hum Pathol* 36, 955-961.
- Sala, A., Kundu, M., Casella, I., Engelhard, A., Calabretta, B., Grasso, L., Paggi, M.G., Giordano, A., Watson, R.J., Khalili, K., *et al.* (1997). Activation of human B-MYB by cyclins. *Proc Natl Acad Sci U S A* 94, 532-536.
- Savage, D.C. (1977). Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 31, 107-133.
- Schauer, M., Janssen, K.P., Rimkus, C., Raggi, M., Feith, M., Friess, H., and Theisen, J. (2010). Microarray-based response prediction in esophageal adenocarcinoma. *Clin Cancer Res* 16, 330-337.
- Schober, F., Neumeier, M., Weigert, J., Wurm, S., Wanninger, J., Schaffler, A., Dada, A., Liebisch, G., Schmitz, G., Aslanidis, C., *et al.* (2007). Low molecular weight adiponectin negatively correlates with the waist circumference and monocytic IL-6 release. *Biochem Biophys Res Commun* 361, 968-973.
- Schulmann, K., Sterian, A., Berki, A., Yin, J., Sato, F., Xu, Y., Olaru, A., Wang, S., Mori, Y., Deacu, E., *et al.* (2005). Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett's-associated neoplastic progression and predicts progression risk. *Oncogene* 24, 4138-4148.
- Scott, C.R., Smith, J.M., Craddock, M.M., and Pihoker, C. (1997). Characteristics of youth-onset noninsulin-dependent diabetes mellitus and insulin-dependent diabetes mellitus at diagnosis. *Pediatrics* 100, 84-91.
- Sellon, R.K., Tonkonogy, S., Schultz, M., Dieleman, L.A., Grenther, W., Balish, E., Rennick, D.M., and Sartor, R.B. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 66, 5224-5231.
- Shapiro, G.I., and Harper, J.W. (1999). Anticancer drug targets: cell cycle and checkpoint control. *J Clin Invest* 104, 1645-1653.
- Sherr, C.J. (2004). Principles of tumor suppression. *Cell* 116, 235-246.
- Shiff, S.J., Shivaprasad, P., and Santini, D.L. (2003). Cyclooxygenase inhibitors: drugs for cancer prevention. *Curr Opin Pharmacol* 3, 352-361.
- Shirvani, V.N., Ouatu-Lascar, R., Kaur, B.S., Omary, M.B., and Triadafilopoulos, G. (2000). Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: Ex vivo induction by bile salts and acid exposure. *Gastroenterology* 118, 487-496.
- Somasundar, P., Riggs, D., Jackson, B., Vona-Davis, L., and McFadden, D.W. (2003). Leptin stimulates esophageal adenocarcinoma growth by nonapoptotic mechanisms. *Am J Surg* 186, 575-578.

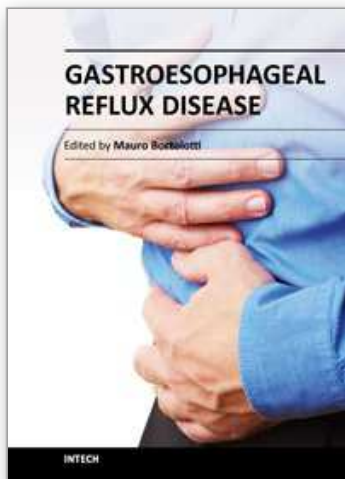
- Song, F., Ito, K., Denning, T.L., Kuninger, D., Papaconstantinou, J., Gourley, W., Klimpel, G., Balish, E., Hokanson, J., and Ernst, P.B. (1999). Expression of the neutrophil chemokine KC in the colon of mice with enterocolitis and by intestinal epithelial cell lines: effects of flora and proinflammatory cytokines. *J Immunol* 162, 2275-2280.
- Sorberg, M., Nyren, O., and Granstrom, M. (2003). Unexpected decrease with age of *Helicobacter pylori* seroprevalence among Swedish blood donors. *J Clin Microbiol* 41, 4038-4042.
- Souza, R.F., Morales, C.P., and Spechler, S.J. (2001). Review article: a conceptual approach to understanding the molecular mechanisms of cancer development in Barrett's oesophagus. *Aliment Pharmacol Ther* 15, 1087-1100.
- Stamatakis, M., Palla, V., Karaikos, I., Xiromeritis, K., Alexiou, I., Pateras, I., and Kontzoglou, K. (2010). Cell cyclins: triggering elements of cancer or not? *World J Surg Oncol* 8, 111.
- Stein, D.J., El-Serag, H.B., Kuczynski, J., Kramer, J.R., and Sampliner, R.E. (2005). The association of body mass index with Barrett's oesophagus. *Aliment Pharmacol Ther* 22, 1005-1010.
- Suerbaum, S. (2009). Microbiome analysis in the esophagus. *Gastroenterology* 137, 419-421.
- Sun, J. (2010). Matrix metalloproteinases and tissue inhibitor of metalloproteinases are essential for the inflammatory response in cancer cells. *J Signal Transduct* 2010, 985132.
- Suzuki, S., Wilson-Kubalek, E.M., Wert, D., Tsao, T.S., and Lee, D.H. (2007). The oligomeric structure of high molecular weight adiponectin. *FEBS Lett* 581, 809-814.
- Sydora, B.C., Tavernini, M.M., Doyle, J.S., and Fedorak, R.N. (2005). Association with selected bacteria does not cause enterocolitis in IL-10 gene-deficient mice despite a systemic immune response. *Dig Dis Sci* 50, 905-913.
- Tashiro, E., Tsuchiya, A., and Imoto, M. (2007). Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. *Cancer Sci* 98, 629-635.
- Tatsuguchi, A., Miyake, K., Gudis, K., Futagami, S., Tsukui, T., Wada, K., Kishida, T., Fukuda, Y., Sugisaki, Y., and Sakamoto, C. (2004). Effect of *Helicobacter pylori* infection on ghrelin expression in human gastric mucosa. *Am J Gastroenterol* 99, 2121-2127.
- Thomadaki, H., and Scorilas, A. (2006). BCL2 family of apoptosis-related genes: functions and clinical implications in cancer. *Crit Rev Clin Lab Sci* 43, 1-67.
- Thrift, A.P., Pandeya, N., Smith, K.J., Green, A.C., Hayward, N.K., Webb, P.M., and Whiteman, D.C. (2011). *Helicobacter pylori* infection and the risks of Barrett's oesophagus: A population-based case-control study. *Int J Cancer*.
- Traganos, F. (2004). Cycling without cyclins. *Cell Cycle* 3, 32-34.
- Trigg, M.E. (1998). Inoculation of a portion of marrow for transplant as a way to accelerate marrow recovery. *Bone Marrow Transplant* 22, 616-617.
- Tummuru, M.K., Cover, T.L., and Blaser, M.J. (1993). Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. *Infect Immun* 61, 1799-1809.
- Vaezi, M.F., and Richter, J.E. (1996). Role of acid and duodenogastroesophageal reflux in gastroesophageal reflux disease. *Gastroenterology* 111, 1192-1199.



- Vaninetti, N.M., Geldenhuys, L., Porter, G.A., Risch, H., Hainaut, P., Guernsey, D.L., and Casson, A.G. (2008). Inducible nitric oxide synthase, nitrotyrosine and p53 mutations in the molecular pathogenesis of Barrett's esophagus and esophageal adenocarcinoma. *Mol Carcinog* 47, 275-285.
- Vieth, M., Schneider-Stock, R., Rohrich, K., May, A., Ell, C., Markwarth, A., Roessner, A., Stolte, M., and Tannapfel, A. (2004). INK4a-ARF alterations in Barrett's epithelium, intraepithelial neoplasia and Barrett's adenocarcinoma. *Virchows Arch* 445, 135-141.
- von Rahden, B.H., Stein, H.J., Feith, M., Puhringer, F., Theisen, J., Siewert, J.R., and Sarbia, M. (2006). Overexpression of TGF-beta1 in esophageal (Barrett's) adenocarcinoma is associated with advanced stage of disease and poor prognosis. *Mol Carcinog* 45, 786-794.
- Vousden, K.H. (2005). Apoptosis. p53 and PUMA: a deadly duo. *Science* 309, 1685-1686.
- Voutilainen, M., Sipponen, P., Mecklin, J-P., Juhola, M., and Färkkilä, M. (2000). Gastroesophageal Reflux Disease: Prevalence, Clinical, Endoscopic and Histopathological Findings in 1,128 Consecutive Patients Referred for Endoscopy due to Dyspeptic and Reflux Symptoms. *Digestion* 61 (1), 6-13.
- Wang, C., Yuan, Y., and Hunt, R.H. (2009a). Helicobacter pylori infection and Barrett's esophagus: a systematic review and meta-analysis. *Am J Gastroenterol* 104, 492-500; quiz 491, 501.
- Wang, D., and DuBois, R.N. (2004). Cyclooxygenase 2-derived prostaglandin E2 regulates the angiogenic switch. *Proc Natl Acad Sci U S A* 101, 415-416.
- Wang, D., Mann, J.R., and DuBois, R.N. (2005). The role of prostaglandins and other eicosanoids in the gastrointestinal tract. *Gastroenterology* 128, 1445-1461.
- Wang, J.S., Guo, M., Montgomery, E.A., Thompson, R.E., Cosby, H., Hicks, L., Wang, S., Herman, J.G., and Canto, M.I. (2009b). DNA promoter hypermethylation of p16 and APC predicts neoplastic progression in Barrett's esophagus. *Am J Gastroenterol* 104, 2153-2160.
- Wang, K.L., Wu, T.T., Choi, I.S., Wang, H., Resetkova, E., Correa, A.M., Hofstetter, W.L., Swisher, S.G., Ajani, J.A., Rashid, A., *et al.* (2007). Expression of epidermal growth factor receptor in esophageal and esophagogastric junction adenocarcinomas: association with poor outcome. *Cancer* 109, 658-667.
- Wani, S., Puli, S.R., Shaheen, N.J., Westhoff, B., Sleghria, S., Bansal, A., Rastogi, A., Sayana, H., and Sharma, P. (2009). Esophageal adenocarcinoma in Barrett's esophagus after endoscopic ablative therapy: a meta-analysis and systematic review. *Am J Gastroenterol* 104, 502-513.
- Ward, J.M., Anver, M.R., Haines, D.C., and Benveniste, R.E. (1994). Chronic active hepatitis in mice caused by Helicobacter hepaticus. *Am J Pathol* 145, 959-968.
- Webb, P.M., Law, M., Varghese, C., Forman, D., Yuan, J.M., Yu, M., Ross, R., Limberg, P.J., Mark, S.D., Taylor, P.R., *et al.* (2001). Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 49, 347-353.
- Whiteman, D.C., Sadeghi, S., Pandeya, N., Smithers, B.M., Gotley, D.C., Bain, C.J., Webb, P.M., and Green, A.C. (2008). Combined effects of obesity, acid reflux and smoking on the risk of adenocarcinomas of the oesophagus. *Gut* 57, 173-180.



- Wilkinson, N.W., Black, J.D., Roukhadze, E., Driscoll, D., Smiley, S., Hoshi, H., Geradts, J., Javle, M., and Brattain, M. (2004). Epidermal growth factor receptor expression correlates with histologic grade in resected esophageal adenocarcinoma. *J Gastrointest Surg* 8, 448-453.
- Wilson, K.T., Fu, S., Ramanujam, K.S., and Meltzer, S.J. (1998). Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. *Cancer Res* 58, 2929-2934.
- Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillon, W.S., Ghatei, M.A., and Bloom, S.R. (2001). Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 86, 5992.
- Wu, A.H., Wan, P., and Bernstein, L. (2001). A multiethnic population-based study of smoking, alcohol and body size and risk of adenocarcinomas of the stomach and esophagus (United States). *Cancer Causes Control* 12, 721-732.
- Wu, X., Gu, J., Wu, T.T., Swisher, S.G., Liao, Z., Correa, A.M., Liu, J., Etzel, C.J., Amos, C.I., Huang, M., *et al.* (2006). Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. *J Clin Oncol* 24, 3789-3798.
- Yang, L., Lu, X., Nossa, C.W., Francois, F., Peek, R.M., and Pei, Z. (2009). Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology* 137, 588-597.
- Yang, L., and Pei, Z. (2006). Bacteria, inflammation, and colon cancer. *World J Gastroenterol* 12, 6741-6746.
- Yarden, Y. (2001). The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. *Eur J Cancer* 37 Suppl 4, S3-8.
- Ye, W., Held, M., Lagergren, J., Engstrand, L., Blot, W.J., McLaughlin, J.K., and Nyren, O. (2004). *Helicobacter pylori* infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. *J Natl Cancer Inst* 96, 388-396.
- Ye, W., and Nyren, O. (2003). Risk of cancers of the oesophagus and stomach by histology or subsite in patients hospitalised for pernicious anaemia. *Gut* 52, 938-941.
- Yildirim, A., Bilici, M., Cayir, K., Yanmaz, V., Yildirim, S., and Tekin, S.B. (2009). Serum adiponectin levels in patients with esophageal cancer. *Jpn J Clin Oncol* 39, 92-96.
- Zhivotovsky, B., and Orrenius, S. (2006). Carcinogenesis and apoptosis: paradigms and paradoxes. *Carcinogenesis* 27, 1939-1945.



## **Gastroesophageal Reflux Disease**

Edited by Prof. Mauro Bortolotti

ISBN 978-953-51-0314-1

Hard cover, 186 pages

**Publisher** InTech

**Published online** 16, March, 2012

**Published in print edition** March, 2012

Gastroesophageal reflux disease affects many patients. This disease not only lowers their quality of life, but it also threatens some of them with an underhand risk of cancer. Additionally, it becomes an economic burden for the patients and society. The aim of this book on gastroesophageal reflux disease is to provide advice and guidance to gastroenterologists to help them understand and manage some aspects of this proteiform disease.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Fritz Francois, Abraham Khan, Liying Yang, Sam M. Serouya and Zhiheng Pei (2012). Gastroesophageal Reflux Disease: Molecular Predictors in Neoplastic Progression of Barrett's Esophagus, Gastroesophageal Reflux Disease, Prof. Mauro Bortolotti (Ed.), ISBN: 978-953-51-0314-1, InTech, Available from: <http://www.intechopen.com/books/gastroesophageal-reflux-disease/molecular-predictors-in-neoplastic-progression-of-barrett-s-esophagus>

**INTech**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen