

## Chapter 2

# CHRONIC INFLAMMATION AND PATHOGENESIS OF GI AND PANCREATIC CANCERS

Lindsey Jackson<sup>1</sup> and B. Mark Evers<sup>1, 2</sup>

*<sup>1</sup>Department of Surgery and <sup>2</sup>The Sealy Center for Cancer Cell Biology  
The University of Texas Medical Branch Galveston, Texas*

**Abstract:** The pathogenesis of cancer represents a complex and multifactorial process requiring a number of acquired and genetic defects. It is becoming increasingly apparent that many cancers originate from a chronic inflammatory process. The topic of this review is the inflammatory response and development of gastrointestinal (GI) and pancreatic cancers. Here, we describe the development of various gastric colorectal and pancreatic cancers through an inflammatory process. The tumor microenvironment which predisposes to tissue destruction, subsequent attempts at healing and accumulation of cellular damage with loss of cell cycle control mechanisms is discussed. Components of the tumor microenvironment that are important in the final common pathway leading to cancer include the tumor stroma, tumor-associated macrophages, cytokines and chemokines and reactive oxygen and nitrogen species. Common signaling pathways that link inflammation with cancer are described and include the COX-2, NF- $\kappa$ B and phosphatidyl inositol 3-kinase (PI3K) pathways. Finally, therapies that can be directed to the inflammatory process as either treatment or prevention of these cancers will be discussed including novel inhibitors of signaling pathways which are currently in development.

**Keywords:** inflammation; carcinogenesis; GI; pancreatitis; inflammatory bowel disease; reactive oxygen species; cytokines; COX-2; NF- $\kappa$ B; PI3K; Akt; PTEN



## 1. INTRODUCTION

The link between chronic inflammation and cancer was first reported by the French surgeon Jean Nicholas Marjolin who, in 1828, described the occurrence of squamous cell carcinoma in a post-traumatic, chronically inflamed wound (Balkwill and Mantovani, 2001). In 1863, Rudolf Virchow identified leukocytes in tumor stroma and suggested that malignancy originated at sites of chronic inflammation, challenging the popular opinion that lymphoreticular infiltrate was simply a reaction to the neoplastic process (Balkwill and Mantovani, 2001). The clinical entity of a cancer arising from a chronic wound is now commonly referred to as a Marjolin's ulcer and can occur in the setting of burn injury, osteomyelitis, venous stasis ulceration, frost bite injury, chronic decubitus ulcers, gunshot wounds, occult trauma, and colostomy sites (Celik *et al.*, 2003). The time between the inciting inflammatory event and the development of cancer is typically 25-40 years but can occur as early as 14 years, with a latency period that is inversely proportional to the patient's age at the time of injury; thus, the younger the patient at the time of injury, the longer the latency period (Celik *et al.*, 2003). Cancer development can be prevented in this setting by early excision of burn wounds or ulcerations and skin grafting to the area. Although far more complicated than treating visible external wounds, perhaps successful elimination of chronic inflammation in other organ systems would have such a preventive effect.

The occurrence of cancers arising after prolonged inflammation has been described in other organs as well (Table 1). Many of these cancers are attributable to infectious, mechanical, or chemical agents that elicit a chronic immune response. For example, mesothelioma, a relatively uncommon form of lung cancer, is associated with asbestos exposure (mechanical agent), and hepatitis B and C infection has a well-defined relationship with the development of hepatocellular carcinoma. It is estimated that 15% of cancers worldwide (approximately 1.2 million cases) are attributable to infectious agents alone (Stoicov *et al.*, 2004). Injury resulting from infections results in cellular proliferation and regeneration which would normally subside once the offending agent was removed or once the tissue was satisfactorily repaired. If the immune response fails to eliminate the inciting agent, proliferation continues in a microenvironment rich in cytokines, growth factors, and accumulating breakdown products of cellular function in a prolonged attempt to repair, often resulting in an accumulation of genetic errors and further inappropriate proliferation. Recent evidence implicates a role for such an inflammatory response in the development of gastrointestinal (GI) and pancreatic cancer, which is the focus of this review.



Table 1. Conditions associated with development of cancer

Inciting agent	Associated cancer
Burn injury →	Marjolin's ulcer
Hepatitis B/C infection →	Hepatocellular carcinoma
HHV8 →	Kaposi's sarcoma
Papillomavirus →	Cervical cancer
Schistosomiasis →	Bladder cancer
Epstein-Barr virus →	Burkitt's lymphoma
Asbestosis →	Mesothelioma
Cigarette smoke →	Lung cancer
Hashimoto's thyroiditis →	Papillary cancer of thyroid

## 2. INFLAMMATION IN GI AND PANCREATIC CANCER

### 2.1 Gastric cancer

While the overall incidence of gastric cancer in the United States has significantly decreased over the past 50 years, it remains the second most common cancer-related mortality in developing countries (Stoicov *et al.*, 2004). The single most identifiable factor contributing to the development of gastric adenocarcinoma is chronic infection with the bacterium *Helicobacter pylori* (*H. pylori*), which has led to its recent classification as a class I carcinogen by the World Health Organization (WHO) (Stoicov *et al.*, 2004). Case-controlled studies have estimated an approximate 2- to 17-fold increased risk of patients seropositive for *H. pylori* to develop gastric cancer when compared with seronegative patients (Stolte and Meining, 1998). A recent Japanese study demonstrated similar results in a cohort of 1,246 patients with documented *H. pylori* infection for approximately 7 years. Of these, 36 patients (3%) developed cancer; individuals with preexisting gastric ulcer or atrophy were at greatest risk (Uemura *et al.*, 2001).

Over half of the world's population is colonized by *H. pylori*, and yet only a small percentage of these individuals will develop gastric disease (Stoicov *et al.*, 2004; Stolte and Meining, 1998). Overall, 15-20% of patients with *H. pylori* infection will develop gastritis or gastric/peptic ulcer disease, while only approximately 1-3% will develop gastric adenocarcinoma or, rarely, non-Hodgkin (MALT) lymphoma of the stomach (Stoicov *et al.*, 2004; Stolte and Meining, 1998). It is apparent that a combination of factors,



which may include bacterial virulence, host genetic, environmental and dietary factors, contributes to the risk of an individual infected with *H. pylori* to develop gastric carcinoma (Stolte and Meining, 1998; Correa, 2003). Such determinants of oncogenic outcome continue to be explored in an effort to identify those individuals at greatest risk for the development of gastric cancer as a result of infection and inflammation. A striking 80% of patients with adenocarcinoma of the stomach are seropositive for *H. pylori* (Stoicov *et al.*, 2004). It is important to distinguish between two histologically distinct forms of noncardia gastric cancer: an intestinal-type and diffuse-type adenocarcinoma. The diffuse type generally affects patients at a younger age and lacks a stepwise progression from premalignant lesion to malignancy. Thought to have a predominantly genetic basis, Parsonnet *et al.* (1991) found that only 32% of patients with this type of adenocarcinoma were *H. pylori* seropositive. The intestinal type generally involves the distal stomach. In contrast to the diffuse type, there is a clear transition from normal mucosa to atrophy, metaplasia, and, eventually, dysplasia, supporting the hypothesis that this form of noncardia gastric cancer arises from a background of chronic inflammation. It is this form most commonly associated with *H. pylori* infection, with approximately 89% seropositivity (Parsonnet *et al.* 1991).

Aggressive treatment of this infection was not initiated until the 1990's, so definitive long-term data on the prevention of gastric cancer by eradication of *H. pylori* is not yet available. However, several studies have shown promising results. Correa *et al.* (2000) performed a nonrandomized chemoprevention trial in a high risk population in Columbia. Individuals received anti-*H. pylori* triple therapy and/or dietary supplementation with ascorbic acid,  $\beta$ -carotene, or placebo; gastric biopsies were performed over a 6-year period. Statistically significant regression of intestinal metaplasia and nonmetaplastic atrophy and prevention of progression was noted in those who underwent triple therapy. Uemura *et al.* (1997) reported that *H. pylori* seropositive patients who underwent early gastric cancer resection with subsequent eradication of the infection demonstrated no recurrence during the 2-year follow-up period, while in a similar group of patients with persistent *H. pylori* infection, almost 9% developed a secondary cancer, further supporting the role of *H. pylori* treatment in gastric cancer prevention. Treatment of *H. pylori* infection is currently reserved only for those with symptomatic dyspepsia or those with confirmed gastritis or peptic ulcer disease, since these individuals are at greatest risk for the development of gastric cancer.



## 2.2 Pancreatic cancer

Pancreatic cancer, the fourth leading cause of cancer death in the US, remains the deadliest of all GI malignancies with an overall five-year survival rate of less than 3% (Farrow *et al.*, 2004; Shi *et al.*, 1999). The propensity for early invasion of local structures and rapid growth contribute to its lethality. Current research and epidemiology indicate a correlation between chronic pancreatitis and the subsequent development of pancreatic cancer, with cancer risk increased 10- to 20-fold in this population (Farrow and Evers, 2002). Hereditary pancreatitis, a rare form of pancreatitis accounting for <1% of all cases, causes wide-spread inflammation and fibrosis affecting the entire organ and is associated with a 53-fold greater risk for cancer development in affected individuals as compared with normal subjects, further supporting such a correlation (Farrow and Evers, 2002).

A large international, multicenter cohort study published in 1993 recruited 1,552 patients from 1946-1989 who fulfilled the diagnostic criteria for chronic pancreatitis and were deemed free of pancreatic cancer for at least 2 years after diagnosis (Lowenfels *et al.*, 1993). Of these subjects, 29 (21 male and 8 female) had evidence of pancreatic cancer two or more years after the diagnosis of pancreatitis was made. The expected number of patients with pancreatic cancer, when adjusted for location, age, and sex was approximately 2, a 17-fold greater incidence in this population with chronic pancreatitis. The effects of several variables on the risk of pancreatic cancer development were compared, including demographic (age, sex, nationality), clinical (comorbidities), and lifestyle variables (smoking, alcohol use), with the only statistically significant predictor proving to be advanced age. This confirmed that the etiology of pancreatic cancer in these individuals was likely the underlying chronic pancreatic inflammation.

## 2.3 Colorectal cancer

Inflammatory bowel disease (IBD), including both ulcerative colitis (UC) and Crohn's disease, has a well established association with the development of colorectal cancer (CRC). In contrast to conditions such as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC), which have a well-defined genetic basis and follow an "adenoma-carcinoma" sequence of development, it appears that chronic inflammation predisposes to the development of CRC in the setting of IBD, following an "inflammation-dysplasia-carcinoma" model (Itzkowitz and Yio, 2004; Lichtenstein, 2002). This is supported by the fact that: 1) anti-inflammatory agents decrease the risk of developing CRC in IBD patients, 2) the risk of



CRC increases with duration of illness, 3) the risk of CRC increases with severity of inflammation, 4) the risk of CRC increases in those patients who demonstrate other inflammatory manifestations of IBD, such as primary sclerosing cholangitis (Itzkowitz and Yio, 2004). Other differences in colorectal cancer development in patients with IBD include a younger age at tumor development, mucinous or signet ring histology, higher incidence of two or more primary tumors, and a more proximal distribution of tumors (Itzkowitz and Yio, 2004).

A large meta-analysis by Eaden *et al.* (2001) identified 116 published studies dating from 1925 that reported incidence of CRC occurring in patients with UC. From these pooled results, they calculated the cumulative probability of a patient with UC to develop cancer by decade of disease, and found incidence rates to be 2% by 10 years, 8% by 20 years, and 18% by 30 years of disease. This approximates the risk cited in a review by Itzkowitz and Yio (Itzkowitz and Yio, 2004), who reported a relative increase in tumor incidence of 0.5-1% per year after seven years of disease. Therefore, patients with UC duration greater than 10 years are at a 20- to 30-fold greater risk of CRC development than the general population (Eaden *et al.*, 2001; Cotran *et al.*, 1999). These estimates likely underestimate true risk as they exclude patients at greatest risk for the development of CRC, since those with the most severe, extensive colitis generally undergo early colectomy.

### **3. CHRONIC INFLAMMATION AND THE TUMOR MICROENVIRONMENT**

The chronic inflammatory response represents a fine balance between active inflammation, repair, and destruction that occurs in response to a persistent stimulus over a prolonged period of time. Activation of leukocytes in response to such an ongoing stimulus leads to the production of chemokines, cytokines, and reactive oxygen species (ROS), resulting in accumulated tissue destruction and subsequent attempts at healing via remodeling, angiogenesis, and connective tissue replacement. Accumulation of cellular damage with loss of cell cycle control mechanisms is thought to be the final common pathway leading to tumor initiation (Balkwill and Mantovani, 2001; Coussens and Werb, 2002).

#### **3.1 Chronic inflammation and tumor stroma**

Tumor stroma is far more likely to contribute to tumor growth, invasion, and immunosuppression than it is to mount an effective anti-tumor response. Pancreatic, gastric, and colorectal cancer stroma all share the common



composition of macrophages, dendritic cells, lymphocytes, fibroblasts, connective tissue, and a fibrin-gel matrix. Farrow *et al.* (2004) compared the inflammatory components of chronic pancreatitis to the tumor stroma associated with pancreatic adenocarcinoma, and subsequently utilized laser-capture microdissection (LCM) to separate stroma from tumor for further analysis. The fibrotic stroma which forms in chronic pancreatitis strongly resembles pancreatic cancer histologically, composed of proliferating fibroblasts, inflammatory cells, and cytokines; only by comparing patterns of gene expression are differences appreciated. Mediators of tumor growth and invasion, such as cyclin E1, the calcium-binding protein S100A4, matrix metalloproteinase 2 (MMP-2), and epidermal growth factor (EGF) were found to be more highly expressed in pancreatic tumor stroma than in chronic pancreatitis. Upon separation of tumor from surrounding stroma, immunohistochemistry demonstrated that while the expression of EGF was higher in the tumor stroma, EGF receptor (EGFR) expression was higher on tumor cells. This suggests a mitogenic relationship between stroma and adjacent tumor, one in which the stroma provides the growing tumor with growth factors and invasion-promoting proteinases. Additionally, NF- $\kappa$ B, a transcription factor whose products regulate oncogenesis, inflammation, and apoptosis, and its activator, I $\kappa$ B kinase, were elevated in both chronic pancreatitis and tumor samples, with staining localized predominantly to either ductal or acinar cells or tumor cells. Upregulation of this protein occurs in response to factors produced by the chronic pancreatitis or tumor stroma, such as IL-8 (9-fold increased expression in chronic pancreatitis as compared to normal tissue) or tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), which provides additional evidence for the existence of a mitogenic relationship.

### 3.1.1 Tumor-associated macrophages

Of the stromal elements, the tumor-associated macrophages are the chief effectors of chronic inflammation in the pathogenesis of pancreatic, gastric, and colon cancer, which produce a large array of inflammatory mediators (Table 2). These inflammatory mediators include growth and angiogenic factors (PDGF, TGF- $\beta$ , EGF), cytokines and chemokines (IL-1, IL-8, and TNF- $\alpha$ ), proteolytic enzymes that degrade the extracellular matrix, promoting invasiveness (proteases, elastase, collagenase, hydrolases, phosphatases, matrix metalloproteinase-9 (MMP-9), and lipases), and cytotoxic agents which likely contribute to host cell genomic damage and promote carcinogenesis (ROS, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), nitric oxide) (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Mantovani *et al.*, 1992). Macrophages also produce migration inhibitory factor (MIF), which



contributes to mononuclear cell immobilization at the site of active, chronic inflammation; however, MIF also has the dual role of suppressing transcriptional activation of the tumor suppressor gene p53, which may also contribute to carcinogenesis (Balkwill and Mantovani, 2001; Farrow and Evers, 2002; Coussens and Werb, 2002).  $H_2O_2$ , a byproduct of macrophage activation, has the ability to activate NF- $\kappa$ B by displacing its inhibitor I $\kappa$ B, leading to translocation of the transcription factor to the nucleus, where it drives the expression of genes regulating inflammation and cell survival, as described in the next section (Farrow and Evers, 2002). TNF- $\alpha$  also activates the NF- $\kappa$ B complex, effectively inhibiting apoptosis (Farrow and Evers, 2002).

*Table 2. Mechanisms by which macrophages contribute to tumorigenesis*

---

A.	Direct DNA damage: reactive oxygen species (ROS)
B.	Bypassing p53, leading to inappropriate cell survival: macrophage inhibiting factor (MIF)
C.	Growth and survival factors: IL-1, IL-6, IL-8, TNF- $\alpha$
D.	Angiogenesis: TNF- $\alpha$ , IL-1, and IL-6 associated with vascular endothelial growth factor (VEGF) production; transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), nitric oxide (NO)
E.	Increased microvascular permeability
F.	Invasion and metastasis: proteases, MMP-9 allow direct invasion; TNF- $\alpha$ and IL-1 augment expression of adhesion molecules on endothelial cells
G.	Subversion of immunity: IL-10 and TGF- $\beta$ are immunosuppressive

---

### 3.1.2 Cytokines and chemokines

TNF- $\alpha$ , IL-1, and IL-6, produced by activated leukocytes, are major mediators of inflammation and tumorigenesis (Cotran *et al.*, 1999; McCawley and Matrisian, 2001; Dvorak, 1986). Together they induce production of adhesion molecules, growth factors, eicosanoids, nitric oxide, chemotactic and angiogenic factors such as VEGF, and are capable of NF- $\kappa$ B and PI3K pathway activation, thus supporting tumor initiation, growth, and invasion. Receptors are found both on stromal elements and tumor cells, suggesting both autocrine and paracrine local effects (Balkwill and Mantovani, 2001; Farrow *et al.*, 2004). Direct evidence for the vital role of TNF- $\alpha$  in tumor development was recently reported in two independent studies (Suganuma *et al.*, 1999; Moore *et al.*, 1999) which found that TNF- $\alpha$  deficient mice were resistant to skin carcinogenesis. In a mouse model of



melanoma, treatment with an IL-1 receptor antagonist significantly decreased tumor growth, and mice deficient in IL-1 $\beta$  were resistant to tumor metastasis (Vidal-Vanaclocha *et al.* 2000). Another study found that IL-6 deficient mice were resistant to chronic inflammation and neoplasia formation in response to intraperitoneal mineral oil injection (Tricot, 2000). The CXC chemokine IL-8 has recently been explored as an important growth and angiogenic factor for pancreatic, gastric, and colorectal tumors. Shi *et al.* (1999) confirmed that inhibition of IL-8 with antisense oligonucleotide *in vivo* suppressed pancreatic tumor growth and metastasis, while increased IL-8 expression increased tumor growth and metastasis.

### 3.1.3 Reactive oxygen and nitrogen species

Neoplasia developing in the setting of chronic inflammation is a multi-hit process, resulting from the accumulation of genetic mutations. These mutations may largely be due to the effects of ROS such as superoxide anions, H<sub>2</sub>O<sub>2</sub>, hydroxyl and hydroperoxyl radicals, and reactive nitrogen species such as NO, peroxynitrite, nitrogen dioxide, and nitrosoperoxy carbonate, collectively known as reactive oxygen and nitrogen species (RONS), that are elaborated by activated inflammatory cells (Stoicov *et al.* 2004; Gasche *et al.* 2001). The toxic effects of RONS include DNA strand breaks, mismatches, mutations, and the formation of adducts with DNA, such as nitrotyrosine (Farrow and Evers, 2002; Hussain *et al.*, 2000). NO is also responsible for the nitrosylation and nitosation of proteins involved in apoptosis, such as caspases-3, -8, and -9, resulting in inactivation and prevention of cell death in response to injury.

To further support the role of RONS in carcinogenesis, Hussain *et al.* (2000) compared the spectrum of p53 mutations in biopsies taken from inflamed and non-inflamed, non-neoplastic colonic mucosa of UC patients to normal, age-matched controls. They noted that greater than half of the inflamed mucosal samples demonstrated p53 mutations that were associated with a concurrent increase in NO synthase-2 (NOS-2) activity, suggesting that oxidative stress was a major contributor. They also noted an increase in post-translational modifications of p53 associated with elevated NOS activity in inflamed samples. Such mutations were not present in non-inflamed mucosal samples. Another study by Gasche *et al.* (2001) examining the effects of H<sub>2</sub>O<sub>2</sub> on *in vitro* colon cancer cell lines demonstrated that H<sub>2</sub>O<sub>2</sub> was capable of damaging the protein complexes responsible for DNA mismatch repair, resulting in inactivation. With higher concentrations of H<sub>2</sub>O<sub>2</sub>, they also demonstrated the presence of frameshift mutations within previously normal mismatch repair genes.



## 4. CELL SIGNALING PATHWAYS THAT LINK INFLAMMATION WITH CANCER

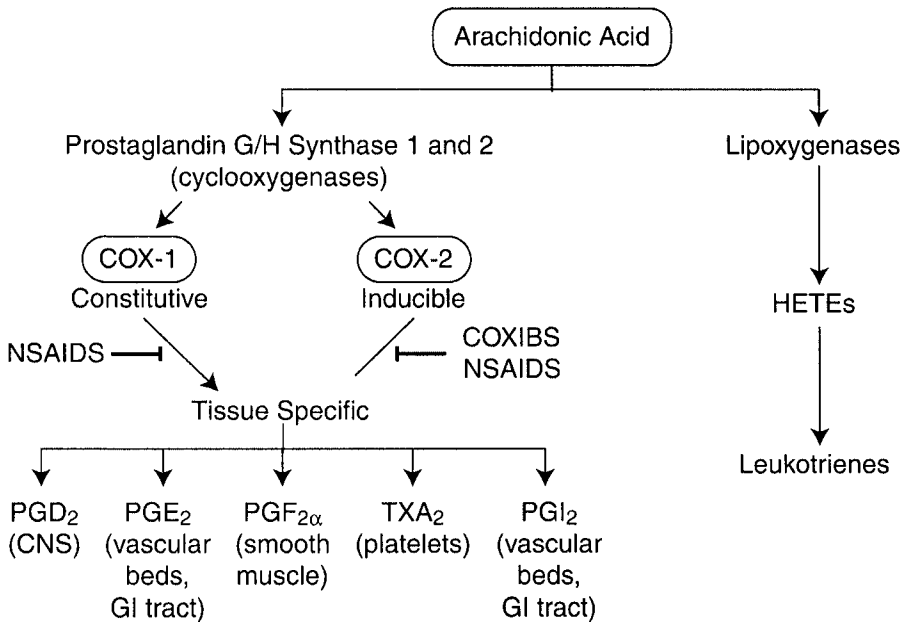
Many derangements in cell signaling occur during the transformation of a normal cell to a malignant phenotype. It is useful to identify cell signaling pathways which may inhibit apoptosis and promote tumor growth which are similarly upregulated in multiple cancer cell lines. Three prominent pathways include the COX-2, NF- $\kappa$ B, and PI3K pathways.

### 4.1 COX-2

Cyclooxygenase (COX), also known as prostaglandin G/H synthase, is the rate-limiting enzyme catalyzing the conversion of arachidonic acid to a variety of inflammatory and physiological mediators, including prostaglandins and thromboxane (Fig. 1). Two isoforms of this enzyme exist which vary in tissue distribution and expression patterns. COX-1 is constitutively expressed in most tissues, and its products regulate homeostatic processes such as platelet function and gastric cytoprotection (Bing *et al.*, 2001; Sheng *et al.*, 1997). In contrast, COX-2 belongs to a class of genes known as immediate early or early growth response genes inducible by inflammatory cytokines and growth factors, including IL-1 and TNF- $\alpha$ , and its products are predominantly pro-inflammatory prostaglandins and eicosanoids involved in regulation of the immune response (Sheng *et al.*, 1997).

COX-2 is not normally expressed in the human intestine, but its activity is significantly elevated in the majority of human colorectal, pancreatic, gastric and other non-GI cancers. A significant and early COX-2 overexpression is associated with UC, both in inflamed and noninflamed mucosa, and with UC-associated dysplasia and neoplasia (Agoff *et al.*, 2000). Similarly, pancreatitis is associated with increased COX-2 expression, and selective genetic deletion of COX-2 or inhibition of its activity significantly reduces the effect of cerulein in the induction of acute pancreatitis and associated acute lung injury in mouse models (Ethridge *et al.*, 2002a). This has led to interest in defining its specific mechanism of action in chronic inflammation and neoplasia to determine if its inhibition may act as an adjunct to current chemotherapy. Large epidemiologic studies have demonstrated a 30-50% reduction in adenomatous polyp formation, incident disease, and death from colorectal cancer by inhibiting COX-2 activity with nonsteroidal anti-inflammatory medications (NSAIDs) (Thun *et al.*, 2002).





*Figure 1.* Arachidonic acid metabolism and the role of COX-1 and COX-2. Tissues of major prostaglandin expression are indicated in parenthesis. PGD<sub>2</sub> = prostaglandin D<sub>2</sub>; PGE<sub>2</sub> = prostaglandin E<sub>2</sub>; PGF<sub>2α</sub> = prostaglandin F<sub>2α</sub>; TXA<sub>2</sub> = thromboxane A<sub>2</sub>; PGI<sub>2</sub> = prostaglandin I<sub>2</sub>, HETE = hydroxyeicosatetraenoic acid

Several mechanisms linking COX-2 overexpression to tumor initiation and progression have been proposed. Two prostaglandin products of COX-2 activity, PGE<sub>2</sub> and PGI<sub>2</sub>, possess angiogenic activity, and are thus believed to support tumor growth and extension. This is supported by the fact that when human or rodent colorectal cancer cells are cocultured with vascular endothelial cells and treated with selective COX-2 inhibitors *in vitro*, migration and tube formation by the endothelial cells can be inhibited (Thun *et al.*, 2002). Agoff *et al.* (2000) suggest two additional mechanisms by which COX-2 activation may promote tumorigenesis - a resultant increase in the derivative malondialdehyde and an up-regulation of the antiapoptotic protein Bcl-2. Malondialdehyde, a genotoxic byproduct of COX-mediated lipid peroxidation and prostaglandin synthesis, has been detected in both sporadic colon cancer and inflammatory bowel disease and is capable of contributing to genomic instability. Bcl-2 inhibits cytochrome c release from the mitochondria and prevents caspase activation, resulting in inhibition of apoptosis. Sun *et al.* (2002) demonstrated that HCT-15 colon cancer cells



overexpressing COX-2 underwent upregulation of the antiapoptotic Bcl-2 protein relative to parental cells, leading to inappropriate cell survival. NSAIDs decrease malondialdehyde levels and Bcl-2 expression, perhaps contributing to the decreased incidence of polyp formation in patients treated with these medications.

Sheng *et al.* (Sheng *et al.*, 1997) observed increased COX-2 mRNA and protein in colonic tumors that developed in rodents exposed to carcinogens. To define the relationship of COX-2 to tumorigenesis, human colon cancer xenografts constitutively expressing COX-2 were implanted into nude mice, and the mice were treated with the highly selective COX-2 inhibitor SC-58125. Mice treated with SC-58125 demonstrated a 90% reduction in tumor development when compared with control mice. Oshima *et al.* (1996) crossed APC<sup>Δ716</sup> mice, known to develop hundreds of intestinal tumors, with COX-2 null mice, and noted an 80-90% reduction in tumor multiplicity in the homozygous COX-2 null offspring. Moreover, a marked reduction in tumor number and size in APC<sup>Δ716</sup> mice treated with a highly selective COX-2 inhibitor was shown, further supporting the role of COX-2 in the development of colorectal cancer.

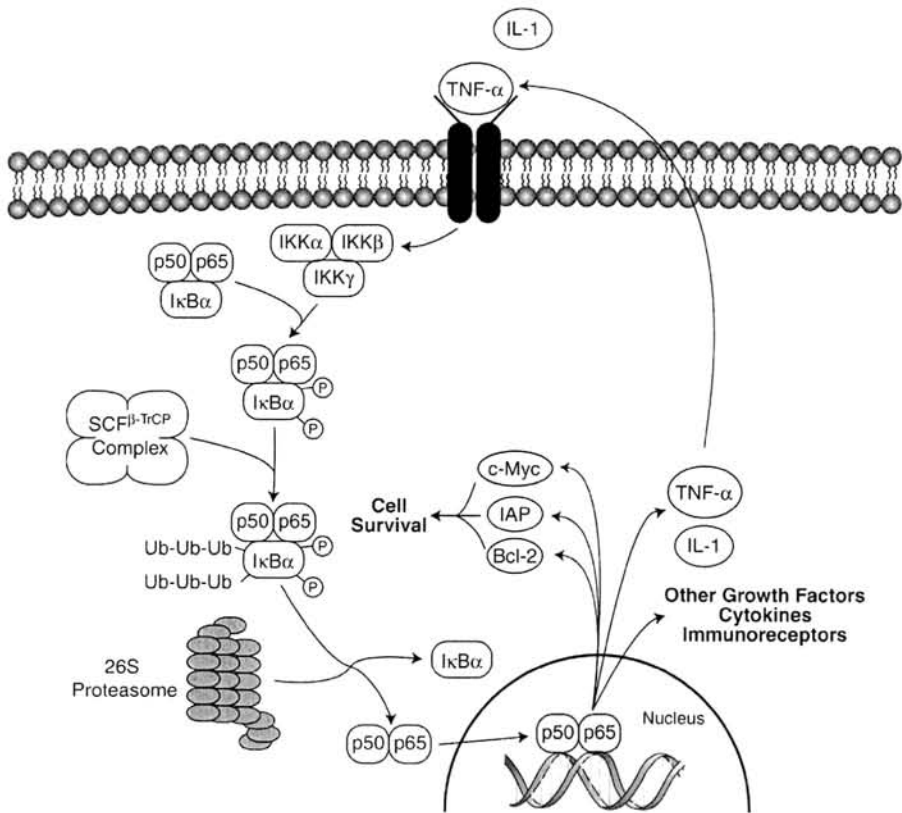
## 4.2 NF-κB

NF-κB is a ubiquitously expressed transcription factor that plays a pivotal role in cellular responses to environmental changes, such as stress, inflammation, and infection. NF-κB is activated in response to infectious agents or cytokines, including TNF-α, IL-1, platelet activating factor (PAF), ROS, lipopolysaccharide (LPS), and leukotriene B4 (Gustin *et al.*, 2004). Its products include growth factors, cytokines, cell adhesion molecules, immunoreceptors, and cell survival proteins, making it an important and complex regulator of the immune response (Gustin *et al.*, 2004; Schwartz *et al.*, 1999). Constitutive activation of NF-κB has been described in inflammatory conditions such as acute and chronic pancreatitis, gastritis, and inflammatory bowel disease, as well as many solid tumors, including GI cancers. The activation of NF-κB by proinflammatory stimuli and its ability to inhibit apoptosis have led to the assumption that the NF-κB pathway provides a mechanistic link between inflammation and cancer (Greten *et al.*, 2004).

The functional NF-κB protein is a heterodimer, most commonly composed of a p65/RelA and a p50 subunit, although many dimers are known to exist *in vivo* (Fig. 2). In a resting cell, NF-κB is bound by inhibitor of NF-κB (IκB) proteins, masking its nuclear localization signal and sequestering inactive NF-κB in the cytoplasm (Baldwin, Jr., 1996; Ethridge *et al.*, 2002b; May and Ghosh, 1998). Stimulation of a cell by an activator



such as  $\text{TNF-}\alpha$  results in a cascade of events, beginning with the activation of the IKK complex, composed of  $\text{IKK}\alpha$ ,  $\text{IKK}\beta$ , and  $\text{IKK}\gamma$ . This complex then phosphorylates  $\text{I}\kappa\text{B}$ , marking it for ubiquitination at specific lysine residues and targeting it to the 26S proteasome. The 26S proteasome degrades  $\text{I}\kappa\text{B}$ , unmasking the nuclear localization signal of  $\text{NF-}\kappa\text{B}$ , thus allowing its translocation to the nucleus where it regulates target gene transcription (Baldwin, Jr., 1996; Ethridge *et al.*, 2002b; May and Ghosh, 1998).



**Figure 2.** The NF- $\kappa$ B pathway. IKK $\alpha$ ,  $\beta$ ,  $\gamma$  = I $\kappa$ B kinase complex; p50, p65 = NF- $\kappa$ B heterodimer; I $\kappa$ B $\alpha$  = inhibitor of  $\kappa$ B; E3-SCF $\beta$ -TrCP complex = ubiquitin ligase complex; Ub = ubiquitination site; P = phosphorylation site; IAP = inhibitor of apoptosis protein.

TNF- $\alpha$  binding to TNF receptor-1 (TNFR1) leads to signal transduction resulting in NF- $\kappa$ B activation and subsequent upregulation of proteins responsible for modulating the immune response and inhibiting apoptosis,



including the *c-myc* proto-oncogene, inhibitor of apoptosis proteins (IAP), and Bcl-2. A positive autoregulatory loop exists whereby some stimulators of NF- $\kappa$ B activation, including TNF- $\alpha$  and IL-1, are upregulated by NF- $\kappa$ B activation, thus potentiating its effect (Kim *et al.*, 2002).

Activated NF- $\kappa$ B has been isolated in macrophages and epithelial cells from biopsy samples and cultured cells of patients with IBD and colorectal cancer, while levels in adjacent normal tissue remain normal. In an effort to further delineate the role of NF- $\kappa$ B specific to the development of colorectal cancer in the setting of chronic inflammation, Greten *et al.* (Greten *et al.*, 2004) examined NF- $\kappa$ B inhibition by deletion of IKK $\beta$  in inflammatory cells compared with intestinal epithelial cells in a mouse model of colitis-associated cancer. They found that deletion of IKK $\beta$  in intestinal epithelial cells was associated with a dramatic decrease in tumor incidence and an increase in tumor cell apoptosis, while deletion in myeloid cells resulted in a decrease in tumor size without an effect on incidence and a reduction in cytokines that serve as tumor growth factors. Luo *et al.* (2004) examined the effect of NF- $\kappa$ B inhibition on the growth and metastasis of a murine model of colon adenocarcinoma. Utilizing an I $\kappa$ B $\alpha$  superrepressor resistant to degradation, they showed that inhibition of NF- $\kappa$ B activation *in vivo* resulted in tumor cell apoptosis and tumor regression. Murano *et al.* (2000) found a significant decrease in cytokines and attenuation of inflammation in mice with dextran sulphate sodium-induced colitis treated with p65 NF- $\kappa$ B subunit antisense oligonucleotide.

Overexpression of NF- $\kappa$ B has similarly been documented in pancreatitis and pancreatic cancer. Wang *et al.* (1999) examined several human pancreatic adenocarcinoma biopsy samples and human pancreatic tumor cell lines and identified constitutive activation of NF- $\kappa$ B in 16 of 24 (67%) pancreatic adenocarcinomas and 9 of 11 (82%) human pancreatic tumor cell lines. Other experiments have documented an increase in NF- $\kappa$ B in mouse and rat models of chemically-induced pancreatitis. Utilizing a mouse model of cerulein-induced pancreatitis, Ethridge *et al.* (2002b) showed that inhibition of NF- $\kappa$ B with NEMO (NF- $\kappa$ B essential modifier = IKK $\gamma$ ) binding domain (NBD) resulted in amelioration of the pancreatitis, thus supporting an essential role for this pathway in the pathogenesis of inflammation and cancer formation.

Interest in the role of NF- $\kappa$ B in tumor development and the role of NF- $\kappa$ B inhibition as an adjunct to chemotherapy and radiation stems from the fact that the transcription factor is activated in many tumors. Chemotherapeutic agents and radiation stimulate NF- $\kappa$ B activation, and this results in the production of anti-apoptotic proteins. Experimental inhibition or deletion of NF- $\kappa$ B attenuated inflammation and tumor growth and



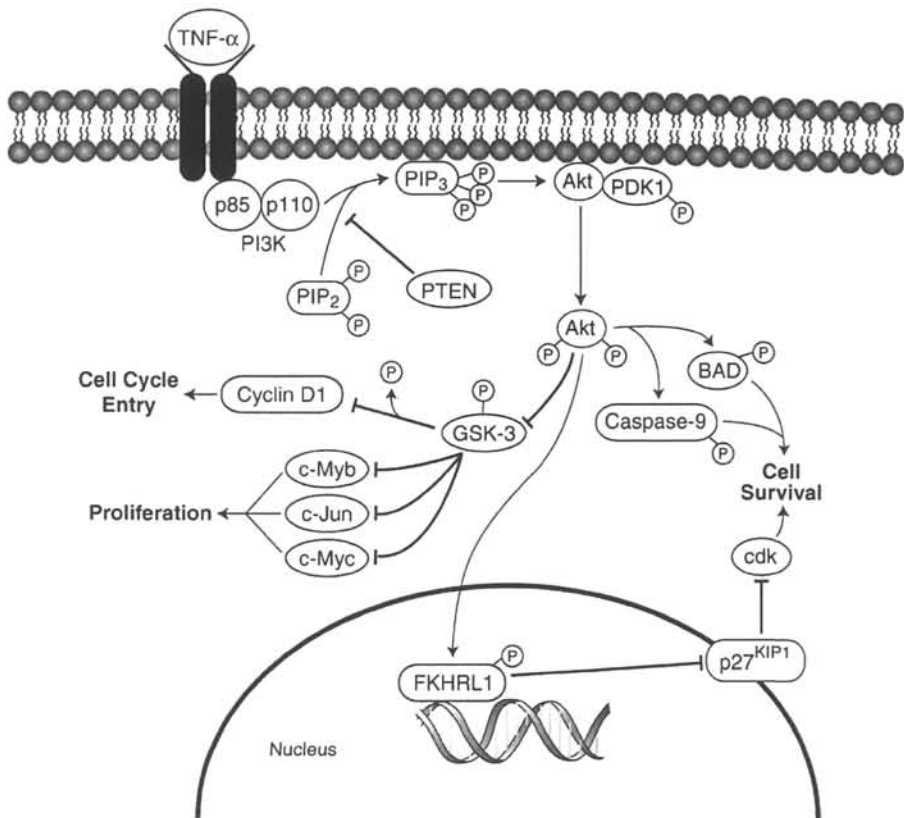
enhanced sensitivity of cancer cells to apoptosis when combined with standard chemotherapeutic agents and radiation.

### 4.3 Phosphatidylinositol 3-kinase (PI3K)

Another pathway playing a critical role in the balance between cell survival and apoptosis is the phosphatidylinositol 3-kinase (PI3K) pathway. PI3K, a ubiquitous lipid kinase activated by a wide variety of extracellular stimuli such as cytokines (e.g., TNF- $\alpha$ ) and growth factors, is involved in the regulation of diverse cellular processes, such as cell growth and survival, actin cytoskeletal rearrangement, membrane ruffling, and vesicular trafficking (Wang *et al.*, 2002a); therefore, signaling through this pathway plays a pivotal role in the regulation of cellular growth, transformation, and tumorigenesis. Increased PI3K activity has been identified in as many as 86% of human colorectal cancers, with increasing activity correlating with increasing tumorigenic potential of the cancer cell lines examined (Wang *et al.*, 2002a; Kaleghpour *et al.*, 2004). The promotion of cell survival by PI3K and its subsequent contribution to tumorigenesis is thought to occur via the inhibition of proapoptotic signals and the induction of survival signals.

PI3K is a heterodimeric protein composed of a regulatory subunit (p85) and a catalytic subunit (p110) (Fig. 3). When its receptor is activated, PI3K catalyzes the phosphorylation of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-phosphate, yielding PIP<sub>2</sub> and PIP<sub>3</sub> (Weaver and Ward, 2001; Wang *et al.*, 2002b; Sheng *et al.*, 2003). PIP<sub>3</sub> then binds protein kinase B (Akt) and phosphatidylinositide-dependent kinase-1 (PDK-1), resulting in their translocation to the plasma membrane, where PDK-1 phosphorylates and activates Akt kinase. Activated Akt kinase then phosphorylates glycogen synthase kinase-3 (GSK-3), rendering it inactive. Constitutively active GSK-3 is unphosphorylated and responsible for maintaining the cell cycle-activating transcription factors *c-myc*, *c-jun*, *c-myb* and cyclin D1 in their inactive (i.e., phosphorylated) state. Inactivation of GSK-3 via phosphorylation therefore allows cells to progress through the cell cycle. Additionally, Akt kinase phosphorylates the Bcl-2 antagonist of cell death (BAD), caspase 9, and forkhead transcription factor (FKHR), suppressing the pro-apoptotic function of these proteins. FKHL1, belonging to a small subset of forkhead family of transcription factors, has the function of regulating p27<sup>KIP1</sup> gene expression when it is in its active, unphosphorylated state. Since the product of the p27<sup>KIP1</sup> gene is a Cdk inhibitor protein, inactivation of this inhibitor via phosphorylation and nuclear exclusion of FKHL1 increases Cdk proteins, thus promoting cell survival (Weaver and Ward, 2001; Wang *et al.*, 2002b; Sheng *et al.*, 2003).





*Figure 3.* The PI3K pathway. p85/p110 = PI3K heterodimer; P = phosphorylation site; PIP<sub>2</sub> = phosphatidylinositol 4,5-phosphate; PIP<sub>3</sub> = phosphatidylinositol 3,4,5-phosphate; PDK = phosphatidylinositide-dependent kinase; GSK-3 = glycogen synthase kinase-3; FKHL1 = forkhead transcription factor; BAD = Bcl-2 antagonist of cell death.

Activation of the PI3K pathway is independently regulated by the tumor suppressor gene PTEN (phosphatase and tensin homolog deleted on chromosome ten), also known as MMAC (mutated in multiple advanced cancers). The product of the PTEN gene, a 3' phosphatase, acts as a tumor suppressor by degrading the PIP<sub>3</sub> product of PI3K activation, thus inhibiting PI3K pathway activation (Weaver and Ward, 2001). Mutation of this gene is associated with several neoplastic disorders, including Cowden's disease, Lhermitte-Duclos disease, and Bannayan-Zonana syndrome, all of which are associated with an increased incidence of colorectal polyposis and cancer, as well as neoplasia in other organ systems (Cantley and Neel, 1999; Kim *et al.*, 2004). PTEN<sup>-/-</sup> knockout mice are embryonic lethals; however, PTEN<sup>+/-</sup>



heterozygous mice, possessing one normal and one defective copy of the PTEN gene, develop intestinal polyposis reminiscent of the human neoplastic disorders (Cantley and Neel, 1999; Kim *et al.*, 2004). It has been shown that restoration of PTEN expression in PTEN<sup>-/-</sup> glioblastoma multiforme, prostate, melanoma, and breast cancer cell lines can lead to tumor growth suppression (Cantley and Neel, 1999), and forced overexpression of PTEN in HT-29 colon cancer cells results in restoration of apoptosis (Arico *et al.*, 2001), supporting the role of PTEN as a tumor suppressor.

The link between the PI3K pathway and carcinogenesis has been explored by examining the differential expression of the various pathway components and the effects of their inhibition or overexpression in tumor tissue. Wang *et al.* (Wang *et al.*, 2002a) demonstrated that PI3K inhibition greatly enhanced sodium butyrate-mediated apoptosis of KM20 human colon cancer cells *in vitro* and *in vivo*, and that the PI3K inhibitor, wortmannin, alone inhibited KM20 xenograft growth *in vivo*. Semba *et al.* (2002) produced similar tumor cell apoptosis and suppression of tumor growth utilizing the PI3K inhibitor, LY294002, in multiple human colon cancer cell lines *in vitro* and *in vivo*. Sheng *et al.* (2003) demonstrated that ectopic expression of either active p110 $\alpha$  or Akt-1 increased rat intestinal epithelial (RIE) cell proliferation *in vitro*, and *in vivo* experiments confirmed that this PI3K activation was associated with increased proliferative activity of the intestinal mucosa which could be attenuated by oral intake of the PI3K inhibitors.

#### 4.4 Summary of pathways

While it has been shown that the COX-2, NF- $\kappa$ B, and PI3K pathways independently contribute to the induction of cell survival signals and the inhibition of apoptosis, a complex relationship exists between the three pathways (Fig. 4). Kim *et al.* (2002) found that treatment of cancer cell lines with TNF- $\alpha$  resulted in NF- $\kappa$ B-dependent transcriptional downregulation of PTEN, leading to activation of the PI3K/Akt pathway. Overexpression of NF- $\kappa$ B-inducing kinase (NIK) or the p65 subunit of NF- $\kappa$ B led to direct increases in phosphorylated Akt and its downstream target, GSK-3 $\beta$ . Ozes *et al.* (1999) noted inhibition of TNF- $\alpha$ -mediated NF- $\kappa$ B activation in cells treated with the PI3K inhibitor wortmannin, dominant-negative PI3K, or kinase-dead Akt. Moreover, Akt-mediated IKK $\alpha$  phosphorylation at threonine 23 was identified as a key interaction between these pathways, since mutation of this amino acid blocks activation of NF- $\kappa$ B. Further evidence for the functional crosstalk between these pathways is the fact that







## 5. IMPLICATIONS FOR FUTURE TREATMENT AND PREVENTION

### 5.1 Anti-inflammatory medications

Many epidemiologic, non-randomized studies have demonstrated a 30-50% reduction in the incidence and mortality from colorectal cancer in individuals taking nonsteroidal anti-inflammatory medications (NSAIDs) on a regular basis, raising interest in the use of these drugs as chemopreventive and chemotherapeutic agents (DuBois *et al.*, 1996; Thun *et al.*, 1991). NSAIDs are classified as either nonselective (affecting both COX-1 and COX-2) or selective (targeting only COX-2). The classes that specifically inhibit COX-2 activity, such as sulindac and celecoxib, are predominantly of interest in the prophylaxis and treatment of colorectal tumors as they decrease the probability of developing the untoward side effects of COX-1 inhibition, including gastritis and peptic ulcer disease.

Tumor inhibition by NSAIDS is believed to occur by at least two distinct cellular processes - the inhibition of angiogenesis and the restoration of apoptosis (Sun *et al.*, 2002). A reduction in two products of COX-2 activation, the proangiogenic factors PGE<sub>2</sub> and PGI<sub>2</sub>, contribute to inhibition of angiogenesis (Ethridge *et al.*, 2002a). Restoration of apoptosis may occur as a direct result of COX inhibition, or through an additional mechanism of action of several NSAIDs (i.e., inhibition of NF- $\kappa$ B). This is thought to occur via inhibition of ATP binding to IKK $\beta$ , thus preventing phosphorylation of I $\kappa$ B and leading to a decrease in NF- $\kappa$ B translocation to the nucleus (Yamamoto and Gaynor, 2001).

The treatment of patients with FAP with NSAIDs has proven to decrease polyp incidence and cause polyp regression, often within the first three months of initiating treatment, and prospective studies on NSAID use in the general population have demonstrated a survival benefit for individuals taking aspirin, a NSAID, on a daily basis (DuBois *et al.*, 1996). Giardiello *et al.* (Giardiello *et al.*, 1993) conducted a double-blind, randomized, placebo-controlled trial of the effects of sulindac on polyp progression in patients with FAP. After 9 months, they found that polyp number had decreased by 44%, and polyp size had decreased by 35% in patients treated with sulindac, while the placebo group experienced an increase in the size and number of polyps. Labayle *et al.* (1991) performed a randomized, double-blind, placebo-controlled trial of sulindac in FAP patients who had had prior colectomy and ileoanal anastomosis. They confirmed a statistically significant decrease in the number of polyps in patients treated with



sulindac, and no change in the placebo group. A prospective study by Thun *et al.* (1991) followed a population of greater than 650,000 persons for 6 years and found that the relative risk of colon cancer mortality among men taking aspirin less than once a month, when controlled for other cancer risk factors, was 0.84, compared to 0.48 for those taking aspirin more than 16 times per month. This represents an approximate 43% decrease in cancer mortality for those taking aspirin 16 times per month or more, reinforcing the potential role of NSAIDs in chemoprevention.

Despite promising results indicating a decreased risk of developing colorectal cancer or a reduction in size of established tumors with NSAID therapy, the potential side effects of administering these medications to healthy individuals as prophylaxis is currently a concern. For example, the COX-2 selective inhibitor valdecoxib (Vioxx) was recently withdrawn from the market after the three-year prospective, randomized, placebo-controlled clinical trial, APPROVe (Adenomatous Polyp Prevention on Vioxx), concluded that there was an increased risk for cardiovascular events (including myocardial infarction and stroke) for those taking the medication (Fitzgerald, 2004). New clinical studies are concentrating instead on the use of NSAIDs as adjuvant therapy for precancerous lesions expressing COX-2 (Thun *et al.*, 2002).

## 5.2 NF- $\kappa$ B inhibitors

The importance of NF- $\kappa$ B to tumor cell survival pathways has identified it as a novel target for cancer chemoprevention. A number of animal studies have demonstrated that experimental inhibition or knockout of NF- $\kappa$ B attenuates tumor growth and enhances the sensitivity of tumor cells to apoptosis when combined with other chemotherapeutic agents (Schwartz *et al.*, 1999; Ethridge *et al.*, 2002b; Luo *et al.*, 2004). Since a number of discrete steps are involved in the activation of NF- $\kappa$ B, as previously discussed, there are several potential methods to down-regulate NF- $\kappa$ B in target tissues. This has led to the development of a wide range of therapeutic agents, including salicylates, corticosteroids, antioxidants, flavonoids (quercetin, resveratrol), proteasome inhibitors (MG-132, -101, and -115, lactacystin, PS-341), synthetic peptides (SN-50), viral proteins (adenoviral E1A protein), nucleotides (antisense oligonucleotides, transcription factor decoys), and plasmids (I $\kappa$ B $\alpha$  expression plasmids), each possessing a different mechanism of action (Schwartz *et al.*, 1999).

Several classes of drugs that inhibit NF- $\kappa$ B either directly or indirectly are currently in clinical use. Glucocorticoids, including prednisone and dexamethasone, widely used as anti-inflammatory and immunosuppressive agents, are capable of inhibiting NF- $\kappa$ B via several different mechanisms.



Dexamethasone induces synthesis of I $\kappa$ B $\alpha$  mRNA, leading to upregulation of I $\kappa$ B $\alpha$  protein and cytoplasmic retention of p65 in monocytes. In endothelial cells, dexamethasone decreases NF- $\kappa$ B transcriptional activity without altering I $\kappa$ B (Thun *et al.*, 1991). Competition between NF- $\kappa$ B and the glucocorticoid receptor for limited amounts of coactivators can also lead to NF- $\kappa$ B inhibition. Sulfasalazine, mesalamine, and sulindac, three anti-inflammatory agents, each have different mechanisms to downregulate NF- $\kappa$ B activation (Yamamoto and Gaynor, 2001). Sulfasalazine, which combines a nonsteroidal moiety (5-aminosalicylic acid or 5-ASA) and an antibacterial moiety (sulfapyridine), is currently used in the treatment of inflammatory bowel disease and rheumatoid arthritis. It inhibits NF- $\kappa$ B activation by suppressing I $\kappa$ B phosphorylation, thus preventing its degradation. Mesalamine prevents p65 phosphorylation without inhibiting I $\kappa$ B degradation. Sulindac is capable of inhibiting IKK $\alpha$  activity by an unknown mechanism, and has proven to induce apoptosis in COX-2 deficient HCT-15 colon cancer cells, thus suggesting that NF- $\kappa$ B, not COX-2 inhibition, is responsible for its effects in this cancer cell line (Yamamoto and Gaynor, 2001). Lastly, the immunosuppressive agents cyclosporine A (CsA) and tacrolimus (FK-506), in addition to their chief mechanism of cell cycle blockage via calcineurin inhibition, are capable of downregulating NF- $\kappa$ B. Cyclosporin acts as a non-competitive inhibitor of the 20S proteasome, preventing I $\kappa$ B degradation, while FK-506 specifically blocks the translocation of the c-Rel subunit of NF- $\kappa$ B to the nucleus (Yamamoto and Gaynor, 2001).

While inhibition of NF- $\kappa$ B alone is unlikely to result in adequate tumor regression, its use as an adjunct with existing chemotherapeutic agents and radiation is promising. Novel approaches to specific NF- $\kappa$ B inhibition continue to be developed, but a lack of specificity and numerous side effects currently limit their usefulness in humans despite promising results in animal studies.

### 5.3 PI3K pathway inhibitors

The importance of the PI3K pathway to cell cycle control, differentiation, and proliferation of tumor cells has led to the development of specific inhibitors targeting the pathway. Like the NF- $\kappa$ B pathway, a cascade of cellular events is responsible for PI3K signaling, and thus there are several intracellular targets for inhibition.

Several potential chemotherapeutic agents that inhibit PI3K activation or interfere with PI3K signaling are currently being evaluated in clinical trials. Rapamycin (sirolimus), a microbially derived antiproliferative agent



currently used as a potent immunosuppressant agent in transplant patients, binds with high affinity to the mammalian target of rapamycin (mTOR), resulting in a decrease in the capacity of cellular translational machinery to meet the increased demand for protein synthesis needed for cell cycle progression (Sekulic *et al.*, 2000). It has recently been discovered that mTOR is normally phosphorylated as a result of PI3K pathway activation; thus, rapamycin exerts its primary effect by inhibiting a downstream effector of PI3K. Phase II clinical trials studying the effects of the rapamycin analogue CCI-779 as a chemotherapeutic agent have been promising. For example, in a recent study by Atkins *et al.* (2004), 26% of 111 patients with renal cell carcinoma refractory to chemotherapy experienced minor responses, seven patients experienced partial responses (at least 50% reduction in cancer load), and one experienced a complete response. Farnesyltransferase inhibitors such as FTI-277 and R115777 induce apoptosis of cancer cells *in vitro* by directly inhibiting PI3K activation, although the precise mechanism remains unclear (Atkins *et al.*, 2004). Clinical trials indicate that the compound R115777 may be the most effective in the treatment of myeloproliferative disorders (two complete responses and two partial responses out of a total of seven patients), while there has been some success with solid malignancies as well (4 partial responses out of 41 patients with metastatic breast cancer treated with R115777) (Brunner *et al.*, 2003). EGFR antagonists directly inhibit activation of PI3K by blocking the receptor's activation in response to TNF- $\alpha$  binding (Mendelsohn and Baselga, 2003). A recent phase II clinical study which combined the EGFR antagonist cetuximab with the chemotherapeutic agent irinotecan in the treatment of advanced colorectal carcinoma in 120 patients resulted in a response rate of 22.5% (Mendelsohn and Baselga, 2003).

Other PI3K inhibitors (wortmannin and LY294002) have been developed, but their use is limited by nonspecific activity and toxicity noted in preclinical trials (Weaver and Ward, 2001; Stein and Waterfield, 2000). Plasmids encoding PTEN or GSK-3 overexpression have been explored, but again, toxicity limits their use (Weaver and Ward, 2001; Talapatra and Thompson, 2001). While promising results have been obtained using PI3K inhibitors *in vitro*, a lack of specificity and systemic toxicity limit their use in the treatment of chronic inflammation and tumorigenesis at this time.

## 5.4 Novel experimental therapies

Conventional chemotherapeutic agents used in the treatment of patients with advanced GI and liver cancer confer limited survival benefit, since many cancer cells resist cell killing by upregulating cell survival pathways or inhibiting cell apoptotic pathways (Bold *et al.*, 1997). Sensitization of such



resistant cells by blocking cell survival pathways or inducing apoptotic mechanisms, used in combination with standard cytotoxic chemotherapeutic agents, has thus become an important focus of cancer research (Schwartz *et al.*, 1999). Such innovative strategies for the treatment of GI cancers include post-transcriptional gene silencing utilizing small interfering RNA (siRNA) or antisense oligonucleotides, anti-cytokine vaccines, transcription factor decoys, and gene expression plasmid transfection (Hasuwa *et al.*, 2002; Tiscornia *et al.*, 2003). Many of these therapies target components of the PI3K, NF- $\kappa$ B, and COX-2 pathways, blocking cell survival pathways and inducing apoptosis when used in combination with standard chemotherapeutic agents and radiotherapy (Talapatra and Thompson, 2001; Hersey and Zhang, 2003). *In vitro* studies utilizing these agents in combination with chemotherapy have been quite promising, but a narrow therapeutic index and a lack of specificity currently limit use for many of them *in vivo* (Hersey and Zhang, 2003). Clinical trials currently in progress using antisense oligonucleotides to Bcl-2 in the treatment of melanoma, chronic lymphatic leukemia, small cell carcinoma of the lung, acute myeloid leukemia, and multiple myeloma, have produced promising initial results with low toxicity (Bold *et al.*, 1997; Hersey and Zhang, 2003). More novel therapeutic agents are expected to enter clinical trial as advances are made in delivery and specificity.

## 6. CONCLUSIONS

Increasing evidence supports the contribution of chronic inflammation to the development of GI and pancreatic cancers. Individuals with chronic inflammatory or infectious diseases (e.g., *H. pylori* gastritis, chronic pancreatitis, IBD) are far more likely to develop malignancy than their healthy counterparts, while treatment of the underlying inflammation or infection has been shown to significantly reduce the risk of cancer development. The production of cytokines, chemokines, and ROS in response to a persistent inflammatory stimulus results in an accumulation of cellular damage. Such stimulators also result in the activation of cell signaling pathways, such as COX-2, NF- $\kappa$ B, and PI3K, which lead to activation of cell survival pathways and inhibition of apoptosis. Although hindered by a lack of tissue specificity and numerous side effects, promising results have already been obtained utilizing novel inhibitors of signaling pathways activated in settings of chronic inflammation and malignancy.



## 7. REFERENCES

- Agoff, S.N. *et al.* (2000). The role of cyclooxygenase 2 in ulcerative colitis-associated neoplasia. *Am. J. Pathol.* **157**: 737-745.
- Arico, S. *et al.* (2001). The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J. Biol. Chem.* **276**: 35243-35246.
- Atkins, M.B. *et al.* (2004). Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J. Clin. Oncol.* **22**: 909-918.
- Baldwin, Jr., A.S. (1996). The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu. Rev. Immunol.* **14**: 649-683.
- Balkwill, F. and Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet* **357**: 539-545.
- Bing, R.J. *et al.* (2001). Nitric oxide, prostanoids, cyclooxygenase, and angiogenesis in colon and breast cancer. *Clin. Cancer Res.* **7**: 3385-3392.
- Bold, R.J. *et al.* (1997). Apoptosis, cancer and cancer therapy. *Surg. Oncol.* **6**: 133-142.
- Brunner, T.B. *et al.* (2003). Farnesyltransferase inhibitors: an overview of the results of preclinical and clinical investigations. *Cancer Res.* **63**: 5656-5668.
- Cantley, L.C. and Neel, B.G. (1999). New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc. Natl Acad. Sci. U. S. A.* **96**: 4240-4245.
- Celik, E. *et al.* (2003). Case report: early arising Marjolin's ulcer in the scalp. *Ann. Burn Fire Dis.* **16**: 217-220.
- Correa, P *et al.* (2000). Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-helicobacter pylori therapy. *J. Natl Cancer Inst.* **92**: 1881-1888.
- Correa, P. (2003). Bacterial infections as a cause of cancer. *J. Natl Cancer. Inst.* **95**: E3.
- Cotran, R.S. *et al.* (1999). *Pathologic Basis of Disease* (W.B. Saunders Company, Philadelphia).
- Coussens, L.M. and Werb, Z. (2002). Inflammation and cancer. *Nature* **420**: 860-867.
- DuBois, R.N. *et al.* (1996). Nonsteroidal anti-inflammatory drugs, eicosanoids, and colorectal cancer prevention. *Gastroenterol. Clin. North Am.* **25**: 773-791.
- Dvorak, H.F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N. Engl. J. Med.* **315**: 1650-1659.
- Eaden, J.A. *et al.* (2001). The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* **48**: 526-535.
- Ethridge, R.T. *et al.* (2002a). Cyclooxygenase-2 gene disruption attenuates the severity of acute pancreatitis and pancreatitis-associated lung injury. *Gastroenterology* **123**: 1311-1322.
- Ethridge, R.T. *et al.* (2002b). Selective inhibition of NF-kappaB attenuates the severity of cerulein-induced acute pancreatitis. *J. Am. Coll. Surg.* **195**: 497-505.
- Farrow, B. and Evers, B.M. (2002). Inflammation and the development of pancreatic cancer. *Surg. Oncol.* **10**: 153-169.
- Farrow, B. *et al.* (2004). Inflammatory mechanisms contributing to pancreatic cancer development. *Ann. Surg.* **239**: 763-769.
- Fitzgerald, G.A. (2004). Coxibs and cardiovascular disease. *N. Engl. J. Med.* **351**: 1709-1711.
- Gasche, C. *et al.* (2001). Oxidative stress increases frameshift mutations in human colorectal cancer cells. *Cancer Res.* **61**: 7444-7448.
- Giardiello, F.M. *et al.* (1993). Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N. Engl. J. Med.* **328**: 1313-1316.



- Greten, F.R. *et al.* (2004). IKK $\beta$  links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118**: 285-296.
- Gustin, J.A. *et al.* (2004). Cell type-specific expression of the IkappaB kinases determines the significance of phosphatidylinositol 3-kinase/Akt signaling to NF-kappa B activation. *J. Biol. Chem.* **279**: 1615-1620.
- Hasuwa, H. *et al.* (2002). Small interfering RNA and gene silencing in transgenic mice and rats. *FEBS Lett.* **532**: 227-230.
- Hersey, P. and Zhang, X.D. (2003). Overcoming resistance of cancer cells to apoptosis. *J. Cell Physiol.* **196**: 9-18.
- Hussain, S.P. *et al.* (2000). Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. *Cancer Res.* **60**: 3333-3337.
- Itzkowitz, S.H. and Yio, X. (2004). Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287**: G7-G17.
- Jobin, C. *et al.* (1998). Specific NF-kappaB blockade selectively inhibits tumour necrosis factor-alpha-induced COX-2 but not constitutive COX-1 gene expression in HT-29 cells. *Immunology* **95**: 537-543.
- Khaleghpour, K. *et al.* (2004). Involvement of the PI 3-kinase signaling pathway in progression of colon adenocarcinoma. *Carcinogenesis* **25**: 241-248.
- Kim, S. *et al.* (2002). PTEN and TNF-alpha regulation of the intestinal-specific Cdx-2 homeobox gene through a PI3K, PKB/Akt, and NF-kappaB-dependent pathway. *Gastroenterology* **123**: 1163-1178.
- Kim, S. *et al.* (2004). Down-regulation of the tumor suppressor PTEN by the tumor necrosis factor-alpha/nuclear factor-kappaB (NF-kappaB)-inducing kinase/NF-kappaB pathway is linked to a default IkappaB-alpha autoregulatory loop. *J. Biol. Chem.* **279**: 4285-4291.
- Labayle, D. *et al.* (1991). Sulindac causes regression of rectal polyps in familial adenomatous polyposis. *Gastroenterology* **101**: 635-639.
- Lichtenstein, G.R. (2002). Reduction of colorectal cancer risk in patients with Crohn's disease. *Rev. Gastroenterol. Disord.* **2** (Suppl. 2): S16-S24.
- Lowenfels, A.B. *et al.* (1993). Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N. Engl. J. Med.* **328**: 1433-1437.
- Luo, J.L. *et al.* (2004). Inhibition of NF-kappaB in cancer cells converts inflammation-induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. *Cancer Cell* **6**: 297-305.
- Mantovani, A. *et al.* (1992). The origin and function of tumor-associated macrophages. *Immunol. Today* **13**: 265-270.
- May, M.J. and Ghosh, S. (1998). Signal transduction through NF-kappa B. *Immunol. Today* **19**: 80-88.
- McCawley, L.J. and Matrisian, L.M. (2001). Tumor progression: defining the soil round the tumor seed. *Curr. Biol.* **11**: R25-R27.
- Mendelsohn, J. and Baselga, J. (2003). Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J. Clin. Oncol.* **21**: 2787-2799.
- Moore, R.J. *et al.* (1999). Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat. Med.* **5**: 828-831.
- Murano, M. *et al.* (2000). Therapeutic effect of intracolonic administered nuclear factor kappa B (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis. *Clin. Exp. Immunol.* **120**: 51-58.



- Oshima, M. *et al.* (1996). Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* **87**: 803-809.
- Ozes, O. N. *et al.* (1999). NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature* **401**: 82-85.
- Parsonnet, J. *et al.* (1991). Helicobacter pylori infection in intestinal- and diffuse-type gastric adenocarcinomas. *J. Natl Cancer Inst.* **83**: 640-643.
- Schwartz, S.A. *et al.* (1999). The role of NF-kappaB/IkappaB proteins in cancer: implications for novel treatment strategies. *Surg. Oncol.* **8**: 143-153.
- Sekulic, A. *et al.* (2000). A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res.* **60**: 3504-3513.
- Semba, S. *et al.* (2002). The in vitro and in vivo effects of 2-(4-morpholinyl)-8-phenyl-chromone (LY294002), a specific inhibitor of phosphatidylinositol 3'-kinase, in human colon cancer cells. *Clin. Cancer Res.* **8**: 1957-1963.
- Shao, J. *et al.* (2000). Regulation of constitutive cyclooxygenase-2 expression in colon carcinoma cells. *J. Biol. Chem.* **275**: 33951-33956.
- Sheng, H. *et al.* (1997). Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J. Clin. Invest.* **99**: 2254-2259.
- Sheng, H. *et al.* (2003). Phosphatidylinositol 3-kinase mediates proliferative signals in intestinal epithelial cells. *Gut* **52**: 1472-1478.
- Shi, Q. *et al.* (1999). Constitutive and inducible interleukin 8 expression by hypoxia and acidosis renders human pancreatic cancer cells more tumorigenic and metastatic. *Clin. Cancer Res.* **5**: 3711-3721.
- Slogoff, M.I. *et al.* (2004). COX-2 inhibition results in alterations in nuclear factor (NF)-kappaB activation but not cytokine production in acute pancreatitis. *J. Gastrointest. Surg.* **8**: 511-519.
- Stein, R.C. and Waterfield, M.D. (2000). PI3-kinase inhibition: a target for drug development? *Mol. Med. Today* **6**: 347-357.
- Stoicov, C. *et al.* (2004). Molecular biology of gastric cancer: Helicobacter infection and gastric adenocarcinoma: bacterial and host factors responsible for altered growth signaling. *Gene* **341**: 1-17.
- Stolte, M. and Meining, A. (1998). Helicobacter pylori and Gastric Cancer. *Oncologist* **3**: 124-128.
- Suganuma, M. *et al.* (1999). Essential role of tumor necrosis factor alpha (TNF-alpha) in tumor promotion as revealed by TNF-alpha-deficient mice. *Cancer Res.* **59**: 4516-4518.
- Sun, Y. *et al.* (2002). Cyclooxygenase-2 overexpression reduces apoptotic susceptibility by inhibiting the cytochrome c-dependent apoptotic pathway in human colon cancer cells. *Cancer Res.* **62**: 6323-6328.
- Talapatra, S. and Thompson, C.B. (2001). Growth factor signaling in cell survival: implications for cancer treatment. *J. Pharmacol. Exp. Ther.* **298**: 873-878.
- Thun, M.J. *et al.* (1991). Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.* **325**: 1593-1596.
- Thun, M.J. *et al.* (2002). Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *J. Natl Cancer Inst.* **94**: 252-266.
- Tiscornia, G. *et al.* (2003). A general method for gene knockdown in mice by using lentiviral vectors expressing small interfering RNA. *Proc. Natl Acad. Sci. U. S. A.* **100**: 1844-1848.
- Tricot, G. (2000). New insights into role of microenvironment in multiple myeloma. *Lancet* **355**: 248-250.



- Uemura, N. *et al.* (1997). Effect of *Helicobacter pylori* eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. *Cancer Epidemiol. Biomarkers Prev.* **6**: 639-642.
- Uemura, N. *et al.* (2001). *Helicobacter pylori* infection and the development of gastric cancer. *N. Engl. J. Med.* **345**: 784-789.
- Vidal-Vanaclocha, F. *et al.* (2000). IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc. Natl Acad. Sci. U. S. A.* **97**: 734-739.
- Wang, Q. *et al.* (2002a). Augmentation of sodium butyrate-induced apoptosis by phosphatidylinositol 3'-kinase inhibition in the KM20 human colon cancer cell line. *Clin. Cancer Res.* **8**: 1940-1947.
- Wang, Q. *et al.* (2002b). Regulation of TRAIL expression by the phosphatidylinositol 3-kinase/Akt/GSK-3 pathway in human colon cancer cells. *J. Biol. Chem.* **277**: 36602-36610.
- Wang, W. *et al.* (1999). The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin. Cancer Res.* **5**: 119-127.
- Weaver, S.A. and Ward, S.G. (2001). Phosphoinositide 3-kinases in the gut: a link between inflammation and cancer? *Trends Mol. Med.* **7**: 455-462.
- Yamamoto, Y. and Gaynor, R.B. (2001). Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. *J. Clin. Invest.* **107**: 135-142.



The Link Between Inflammation and Cancer

Wounds that do not heal

Dalgleish, A.G.; Haefner, B. (Eds.)

2006, XII, 254 p., Hardcover

ISBN: 978-0-387-26282-6