II. LITERATURE REVIEWS

1. Anatomy and histology of the stomach and large bowel

The stomach is the part of the digestive system that lies between the lower end of the esophagus and the beginning of the small intestine. Once food is swallowed it passes down the esophagus and into the stomach that functions as an antechamber of the gut. Its proximal portion, consisting of the body and fundus, not only act as a reservoir in which ingested food is stored but also serves as a decontamination chamber in which biological hazards are destroyed by caustic acid and pepsin. Its distal portion, the antrum, is a muscular grinding chamber that pulverized solid food and feeds it gradually into the digestive process.

The large bowel or colon and rectum is a storage and absorptive organ. It starts in the right lower abdominal fossa where the small intestine enters through the ileocecal valve. This part is called the cecum. The appendix is attached to the lower part of the cecum. The next part is called the ascending colon, which starts up to the hepatic flexure also called the right flexure situated just under the liver in the right upper abdominal quadrant. The transverse colon runs transversely across the abdomen to the splenic flexure also called the left flexure lying just below the spleen in the left upper abdominal quadrant. The colon runs downward, which is called the descending colon and downs to the left lower abdominal quadrant where it makes an S-shaped loop called the sigmoid colon. The sigmoid colon runs down into the pelvis and the rectum. The junction between the sigmoid colon and the rectum is called the rectosigmoid junction. Rectum then goes over into the anal canal that ends with the anus. The diagram illustrates the position of the stomach and large bowel is shown in Figure 1.

The wall of the gastrointestinal tract has four concentric layers (Fig. 2). Starting from the lumen, mucosa is closest to the lumen and consists of the epithelium (simple columnar), lamina propria, and muscularis mucosae. Under the mucosa comes the submucosa. Then comes the muscularis propria and then comes the serosa which is the external coat consisting of a layer of mesothelial cells resting on loose connective tissue outside the muscular layer. The lower part of the rectum lacks the serosa as it goes down and leaves the peritoneal cavity.

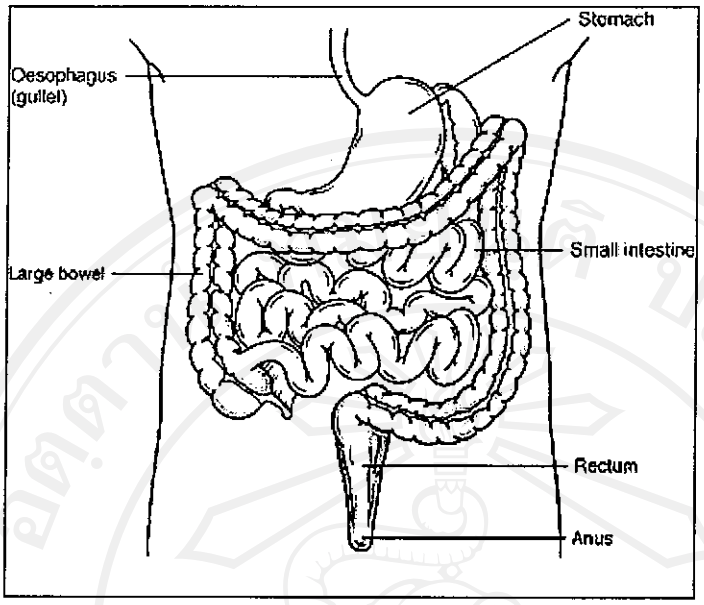

Figure 1. The position diagram of the stomach and large bowel.

{CancerBACUP. 2003. "Understanding cancer of the stomach." [Online]. Available http:// / www. cancerbacup. org. uk (18 June 2003).}

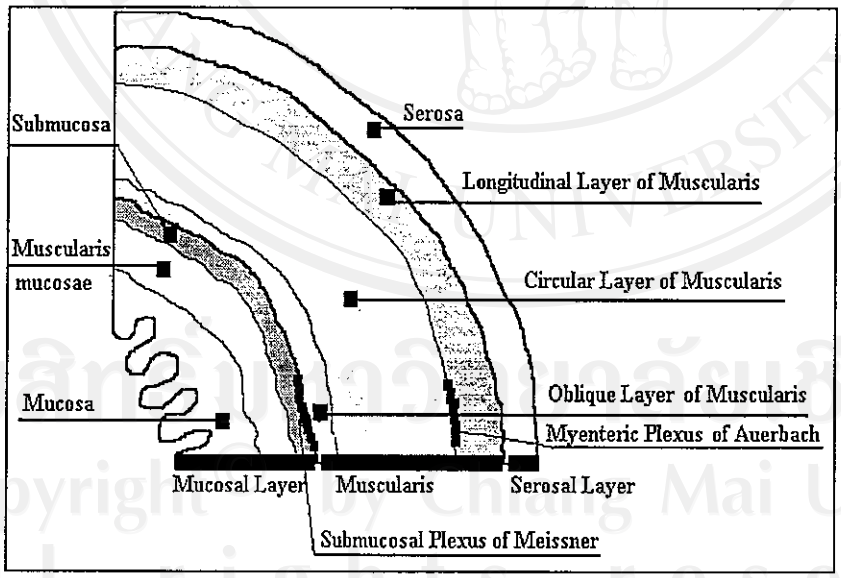

Figure 2. Layers of the gastrointestinal tract wall.

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2. Stomach cancer

Normally, the word cancer is a popular diction for a malignant neoplasm. Cancers arising in epithelial cells, which are the cells that outline the hollow organs, are called carcinomas. While cancers arising in the mesenchymal tissues (non-epithelial cells), are called sarcomas. Carcinomas are divided further into adenocarcinomas when they have a glandular growth pattern and squamous cell carcinomas when they produce recognizable squamous cells. It's further practice to specify the organ of origin, such as stomach or gastric adenocarcinoma. These are more than 200 different kinds of cancer, each with its own name and treatment.

Stomach cancer is a malignant neoplasm of the stomach. It is one of the most common malignancies in the world, especially in Eastern Asia including Korea and Japan (Ahn *et al.*, 1991). Approximately, 90% of stomach cancers are adenocarcinomas and the remaining 10% are Non-Hodgkin's lymphomas and leiomyosarcomas (Kelley and Duggan, 2003). Whereas adenosquamous, squamous, and undifferentiated carcinomas are rarely occured. Other very rare primary malignant neoplasms of the stomach include choriocarcinomas, carcinoid tumors, rhabdomyosarcomas, and hemangiopericytomas. Kaposi's sarcomas, in association with the acquired immunodeficiency syndrome, have also been reported (Jass, 1992). Moreover, adenocarcinomas may be divided histologically into two general subtypes by the Lauren classification, including a well differentiated or intestinal type, with cohesive neoplastic cells forming gland-like structures that frequently ulcerate, has a better prognosis and a poorly-differentiated diffuse or diffuse type in which cell cohesion is absent, resulting in infiltration and thickening of the stomach wall without the formation of a discrete mass (Lauren, 1997). Since different combinations of genetic changes have been found in these two histologically distinct types of stomach cancer, they may possess different genetic backgrounds (Stemmermann *et al.*, 1994; Tahara *et al.*, 1996).

It is known that cancers can be caused by genetic alterations that turn on oncogenes or turn off tumor suppressor genes. Loss of or damage to these genes can also lead to some cancers. In stomach cancer, most of the genetic changes that lead to the cancer occur after birth. While inherited genetic changes account for a slight percentage of stomach cancer. Although a molecular pathogenesis between stomach adenoma and differentiated adenocarcinoma has not been formally established so far (Tamura, 1996). However, several studies indicated that the

molecular alterations, the most well known genetic mutations including translocated promoter region-*MET* (TPR-*MET*) rearrangement, alterations of K-*ras*, *p*53 and *APC* gene, as well as loss of heterozygosity (LOH) of the deleted in colorectal cancer (*DCC*) gene, potentially involved in stomach carcinogenesis (Correa and Shiao, 1994; Dijkhuizen *et al.*, 1997; Shiao *et al.*, 1998).

Interestingly, a recent study performed on 19 stomach cancer patients and 25 controls suggested that TPR-*MET* activation might be an early event in stomach carcinogenesis (Roth, 2003). Another study performed on 137 participants in very high-risk populations in Colombia indicated that the presence of K-*ras* mutation predicted the progression of preneoplastic stomach lesions to carcinoma in the subsequent years (Gong *et al.*, 1999). Likewise, some reports showed that the mutation of the p53 gene was an early event in stomach tumorigenesis (Shiao *et al.*, 1994). Furthermore, microsatellite instability (MSI) and LOH at several loci may also participate in the progressive development of some stomach neoplasms (Strickler *et al.*, 1994; Chong *et al.*, 1994; Kobayashi *et al.*, 2000; Palli *et al.*, 2001) and some data on small cohort events suggested that the presence of MSI in chronic gastritis or in hyperplasic and adenomatous polyps of the stomach could be a predictor for progression to gastric adenocarcinoma (Nogueira *et al.*, 1999; Kashiwagi *et al.*, 2000; Leung *et al.*, 2000).

3. Risk factors for stomach cancer

A risk factor is anything that increases a person's chance of getting a disease such as cancer and different cancer type has different risk factors. While a cause of stomach cancer is not known exactly, scientists have found several risk factors that make a person more likely to develop stomach cancer, as follow:

a. *Helicobacter pylori* (HP) infection

Since HP was first discovered by Warren and Marshall in 1983, a number of evidence has been gathered concerning this organism and its role in the etiology of stomach cancer (Warren and Marshall, 1983). There are many studies to support a causal association between HP and stomach cancer. Some studies suggested that the risk of stomach cancer was increased two- or three-folds in those chronically infected with HP (Danesh, 1999; Eslick *et al.*, 1999). Therefore, HP contributes to the causation of stomach cancer, present in the great majority of

cases of this cancer, via mechanisms that include the development and progression of chronic gastritis (Sipppnen *et al.*, 1994; Valle *et al.*, 1996).

b. Dietary factors

An increased risk of stomach cancer is associated with diets containing large amounts of smoked foods, salted fish and meat, certain foods high in starch that are also low in fiber, and pickled vegetables. Additionally, nitrites and nitrates are substances usually found in certain vegetables, preserved meats, and some drinking water. Dietary nitrite or nitrate can be converted into *N*-nitroso compounds in the human stomach that such compounds have been shown to cause stomach cancer in animal experiments (Joossens *et al.*, 1996). In contrast, eating whole grain products, fresh fruits and vegetables that contain vitamin C (ascorbate) appears to lower the risk of stomach cancer (Kono and Hirohata, 1996; Neugut *et al.*, 1996).

c. Smoking and alcohol

The relationship between smoking and stomach cancer has been extensively examined but remains unclear. While most studies have reported a weak to moderate association, a few have found none (Kelley and Duggan, 2003). In addition, there are some studies that have linked an association between alcohol consumption and stomach cancer, this is not certain as well.

d. Stomach surgery

Stomach cancers are more likely to develop in patients who have had part of their stomach removed to treat noncancerous disease such as ulcers (Stalnikowicz and Benbassat, 1990). Furthermore, there are many studies that include large long-term follow-up studies point to an increased risk of stomach cancer particularly 15 years or more after the surgery (Tersmette *et al.*, 1990; Fisher *et al.*, 1993; Tersmette *et al.*, 1995; Molloy and Sannenberg, 1997).

e. Pernicious anemia

Certain cell in the stomach lining also produces a substance, which helps to absorp vitamin B12 from foods. If the substance is not enough present, a vitamin B12 deficiency may occur causing problems in the development of red blood cells that is called anemia. The relationship between pernicious anemia and stomach cancer has long been recognized. Hsing *et al.* reported that there was three-fold increased in the risk of stomach cancer in a cohort of 4,517 patients with pernicious anemia, followed for up to 20 years (Hsing *et al.*, 1993).

f. Familial cancer syndrome

Although the contribution of hereditary factors to the causation of sporadic cancer is unclear, some studies have reported hereditary nonpolyposis colorectal cancer (HNPCC) and FAP which are inherited genetic disorders cause a greatly increased risk of developing colorectal cancer and a slightly increased risk of stomach cancer in family member affected by these inherited gene mutations (Lichtenstein *et al.*, 2000).

g. Other risk factors

Stomach cancer is about twice as common in men as it is in woman and has a sharp increase after the age of 50. In addition, the risk of stomach cancer is increased in first-degree relatives of patients with the disease by about two-to three-fold (La *et al.*, 1992; Palli *et al.*, 1994; Lissowska *et al.*, 1999). Other suggested risk factors include blood group A, stomach polyps, and geography. These factors can cause an increased risk of stomach cancer but the reason is unknown.

4. Colorectal cancer

Colorectal cancer is the third most common cancer and the second most common cause of death from cancer in many industrialized countries. It arises from the epithelial cells outlining the lumen of the colon and rectum. The cancer is thus called a colorectal adenocarcinoma that regard to the most histological type of all colorectal cancer. There are other forms of colorectal cancer such as squamous cell carcinoma, sarcoma, lymphoma, and carcinoid tumors, which are very rare and altogether constitute less than 2% of all this cancer. They are often not treated the same way as adenocarcinoma. In histologic grading, as in the system of Broders (Broders, 1926), most investigators use a numbering system from 1 to 4, with larger numbers indicating less differentiation, or a series of modifying terms designating tumors as well, moderately or poorly differentiated (Dukes, 1950) which poorly differentiated carcinomas will carry a less favorable prognosis than do more differentiated carcinomas.

Besides grade of tumor, pathological features including lymphatic, vascular and perineural infiltration, and presence or absence of an inflammatory response related to prognosis as well. About 20% of all colorectal cancers are estimated to be hereditary which include familial colorectal cancer accounts for about 15%, HNPCC for about 5%, and FAP for about 1%. There are also other very rare syndromes as Juvenile polyposis, Gardner's syndrome, Turcot's

syndrome, and Peutz-Jeghers syndrome. On the other hand, about 80% remaining are non-hereditary colorectal cancer that is called sporadic.

Several studies indicated that certain genetic factors, environmental factors, or dietary factors were probably involved in the etiology of colorectal cancer. Research in epidemiological, clinical and genetic evidence found that a great number of colorectal adenocarcinomas developed from a benign adenomatous polyp progressing though a sequence of events, which might take about 15-20 years (Winawer *et al.*, 1996).

The progression of events leading to the transformation of colonic epithelial cells into a cancer is multistep process that starts with stepwise accumulation of multiple genetic defects (Kinzler and Vogelstein, 1998). There may be as many as twenty genes involved in colorectal carcinogenesis. Especially, the most well defined genetic mutations include these in the *APC* gene on chromosome 5, *K-ras* on chromosome 12, *DCC* on chromosome 18, and *p*53 on chromosome 17 (Fig. 3). Interestingly, mutation of the *APC* tumor suppressor gene has been detected in 36% to 79% of patients with colorectal cancers and adenomas (Vogelstein *et al.*, 1988; miyoshi *et al.*, 1992), suggesting that an *APC* gene mutation may be an early or initiating event in the colorectal tumorigenesis. In this way, the first an aberrant crypt foci is formed.

Additionally, genetic events involve the formation of a very small polyp (adenoma) that the latter turns into a cancer as a result from accumulation of these distinct genetic alterations. It has been show that the conversion of late colorectal adenoma to carcinoma was associated with genetic changes of both *p*53 alleles (Kikuchi-Yanoshita *et al.*, 1992), indicating that *p*53 is important for the suppression of cellular transformation. *p*53 is an essential protein for the regulation of genome integrity. Every day of our life, cells face many dangers, including chemical, viruses and ionizing radiation, which can damage the genome and lead to cancer development. *p*53 binds to many regulatory sites in the genome and arrest cell division until the damage is repaired. Or, if the damage is too severe, *p*53 initiates the process of apoptosis (Clarke *et al.*, 1993), which directs the cell to commit suicide, permanently removing the damage. Therefore, loss of *p*53 function will result in a massive accumulation of mutations and instability of the cellular genome, which is the main character of malignant phenotype. This explains why loss of *p*53 function can converse colorectal adenoma to colorectal carcinoma. Thus, these events

require multiple genetic alterations with activation of oncogenes and inactivation of tumor suppressor genes that are essential for the tumorogenesis.

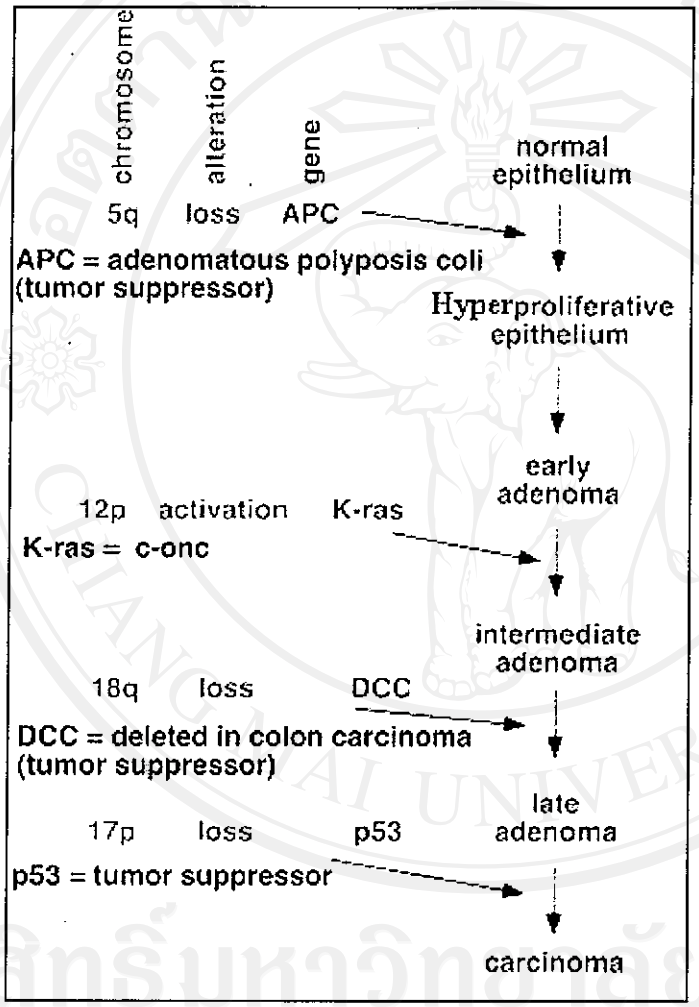

Figure 3. Molecular model for the progression of colorectal cancer through the adenoma-carcinoma sequence (Modified from Kinzler KW, Vogelstein B. A genetic model for colorectal tumorogenesis. Cell 1996; 87; 159-170.).

5. Risk factors for colorectal cancer

The specific causes of colorectal cancer are unknown, but environmental, nutritional, genetic and familial factors and previous existing diseases have been found to be associated with this cancer. However, there have been several epidemiological studies that reveal the relationship between many risk factors and colorectal cancer, as follow:

a. Dietary factors

Diets rich in fat and cholesterol have been related to an increased risk of colorectal cancer (Steele, 1994). A sedentary lifestyle and obesity, both linked with dietary fat, also correlated with the incidence of this cancer (West *et al.*, 1989; Kune *et al.*, 1990; Lee *et al.*, 1991). Moreover, dietary fat increases the endogenous production, degradation of bacteria, and bile acids and neutral steroids excretion that are carcinogens able to promote colonic carcinogenesis (Calabresi, 1993). Excess lipids in the colon may cause an increase of the concentration of secondary bile acids, which may stimulate protein kinase C (PKC), a major cellular communication pathway, resulting in the promotion of the cancer (Morotomi *et al.*, 1990). Additionally, a diet high in fat can lead to a predominance of anaerobic bacteria in the intestinal microflora and the enzymes in such bacteria may activate carcinogens (Nigro *et al.*, 1975; Reddy *et al.*, 1976; Wargovich and Felkner, 1983). On the other hand, a protective effect has been suggested for a diet containing fiber and green and yellow vegetables (Weisburger, 1991).

Possible effects of the fiber on colorectal tumorigenesis may include decreased exposure to fecal carcinogens, reduced carcinogenic microflora in the bowel, and decreased fecal pH with a consequent decrease in bacterial enzymatic activity and a dilution of carcinogens via an increase in stool bulk (Burkitt *et al.*, 1974; Walker *et al.*, 1986; Cummings and Bingham, 1987). Additionally, diet compounds that may decrease colorectal tumorigenesis are vitamin C, D and E, indoles, beta-carotene, and selenium (Schober, 1986; Potter and McMichael, 1986; Wargovich *et al.*, 1988).

b. Familial factors

The majority of genetic premalignant polyposis syndromes have been described, including familial colorectal cancer, HNPCC, and FAP. Although if none of these syndromes are present, patients with family histories of colorectal cancer still have an increased risk for the

disease (Macklin, 1960). Fuchs *et al.* confirmed this in the first prospective study of 32,085 men and 87,031 women who were first-degree relatives of colorectal cancer patients and indicated that the risk was more evident in younger people (Fuchs *et al.*, 1994).

In familial colorectal cancer the causative genes have not been characterized. Individuals have an increased risk of the cancer which if one or more first-degree relatives have developed colorectal cancer, especially if it was diagnosed at a young age. Whereas, the best characterized conditions that inherit a clear genetic predisposition to colorectal cancer have been grouped into either nonpolyposis or polyposis syndromes based on the phenotype they exhibit (Kinzler and Vogelstein, 1996). These conditions are HNPCC and FAP.

There are many investigations reported HNPCC, some families without a history of adenomatous polyps in the large bowel, also have a higher risk of developing colorectal cancer (Lynch and Lynch, 1978; Lynch *et al.*, 1983). It is inherited as an autosomal domonant trait with more than 90% penetrance (Macklin, 1960) and caused by defects of mismatch repair genes, which the most common mutations arise on *hMSH2* and *hMLH1* gene. Patients with HNPCC typically have very few polyps and often develop carcinomas on the right side of the colon (60% to 70%) at an early age of onset (median, 44 years) (Vasen, 1994).

Otherwise, FAP is also an autosomal dominantly inherited trait. It is important because affected individuals carry an almost 100% lifetime risk of getting colorectal cancer. It is caused by mutation in the *APC* gene, located on chromosome 5, which is a tumor suppressor gene and inactivation of this gene leads to neoplastic growth. Multiple adenomatous polyps, more than hundreds to thousands, develop in the large bowel during the teenage of early adulthood and if not discovered and treated colorectal cancer develops in almost 100% most often the age of 40.

c. Inflammatory bowel disease

Patients with inflammatory bowel disease such as Crohn's disease and ulcerative colitis have a higher incidence of colorectal cancer than normal. Moreover, the risk of carcinoma in patients with ulcerative colitis is associated with the duration of active disease, extent of colitis, development of mucosal dysplasia and continuity of symptoms (Edwards and Truelove, 1964; Morson, 1966; Lee and Truelove, 1980; Butt and Morson, 1981). Likewise, the risk of developing colorectal cancer also is increased in Crohn's disease which a lesser extent than in patients with ulcerative colitis.

d. Colorectal polyps

Colorectal cancers develop more often in patients who had adenomatous polyps than in those without polyps. About 5% probability of carcinoma will be present in an adenoma and the risk correlates with the histology and size of the polyp. In 1993, Winawer *et al.* concluded that colonoscopy-guided polypectomy decreased the incidence of unexpected colorectal cancer. This supported the view that colorectal adenoma progress to carcinoma (Winawer *et al.*, 1993).

e. Cancer history

There are some studies found that women with a history of breast, endometrial, or ovarian cancer also have an increased chance of developing colorectal cancer (McGregor and Bacon, 1958). Furthermore, patients with a history of colorectal cancer have an increased risk of developing a second primary colon cancer or other malignancy (Schottenfield *et al.*, 1969).

f. Other risk factors

Other suggestions for the risk factors including sedentary occupations (Ballrad-Barbash *et al.*, 1990) and history of pelvic irradiation for gynecologic cancer (Weiss *et al.*, 1981) all have related to a higher risk of colorectal cancer.

In Thailand, a country with high incidence of colorectal and stomach cancers, dietary factors and dietary habits are crucial risks, which probably play an important role in development of the tumors (Hart *et al.*, 1993; Sriamporn *et al.*, 2002). For example, cured meats and fermented porks which frequently consumed in Thai contain nitrates and nitrites substances and subsequently can be converted into *N*-nitroso compounds in the stomach, resulting in an increase risk of developing colorectal cancer (Lohsoonthhorn and Danvivat, 1995). Moreover, nowadays Thai people are changing their lifestyle to consume more fast foods, which are rich in fat and cholesterol, one of the risk factors for GI tract cancer. Additionally, it has been demonstrated that more than seventy percent of Thai patients with gastroduodenal diseases are the *Helicobacter pylori* carriers who have serum antibodies to Cag A seroprevalence (Mahachai *et al.*, 1990), indicating that there is high incidence of *Helicobacter pylori* infection, another risk factor for stomach cancer, among Thai population.

6. Staging of stomach and colorectal cancer

The stage of these cancers is not finally revealed until after the resection when the removed specimen has been analyzed by the pathologist. It is a process that tells the physician how widespread a cancer may be and this will show if the cancer has spread and how far. The treatment and prognosis for the cancer depend, to a large extent, on the patient's stage at diagnosis.

The stage system for stomach and colorectal cancer used in the United Stages is the international TNM system developed by American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) (Fleming *et al.*, 1997). The characteristics that form the basic of the staging system are based on the assessment of three components including T stands for the extent of the primary tumor, N stands for the absence or presence and extent of regional lymph node metastasis, and M is for the absence or presence of distant metastasis. The use of numerical subsets of the TNM component indicates the progressive extent of the malignant disease. In TNM staging, information about the tumor, lymph nodes, and metastasis is compound in a process called stage grouping (Table 1).

Additionally, the main staging systems, which except the TNM, for colorectal cancer are the Dukes system (Dukes, 1932; Zinkin, 1983). The Dukes pathologic staging system separates colorectal malignancies into five groups. Lesions confined to the bowel wall and not penetrating the muscularis are designated A, lesions penetrating the muscularis into surrounding fat or adventitia are designated B, lesions with positive lymph node involvement are designated C, and D stage for patients with metastasis. The detail of these systems for colorectal cancer staging as well as a comparison with each other appeared in Table 2.

7. Cyclooxygenase isoenzymes

The COX enzyme are a key enzyme in the biosynthetic pathway leading to formation of PGs which are potent biological mediators with diverse normal physiological effects and are also implicated in a variety of pathological conditions including inflammation and tumorigenesis (Watkin *et al.*, 1989; Dewitt, 1991; Xie *et al.*, 1992). The enzymes have been identified into two isoforms, COX-1 and COX-2. COX-1 was first purified from bovine vesicular glands in 1976 (Miyamoto *et al.*, 1976) and cloned in 1988 (DeWitt and Smith, 1988). It is constitutively expressed in most tissues including kidney, lung, stomach, duodenum, jejunum, ileum, colon and

cecum of rat, dog, Rhesus monkey, and human (Kargman *et al.*, 1996) and has been proposed to generate PGs, such as PGE_2 and PGI_2, which are thought to be critical to normal physiological functions (Smith, 2000). In the early 1990s, a second isoform, now known as COX-2, was discovered. It is very similar in structure and catalytic activity with COX-1. It was found that the biosynthetic activity of both isoforms can be inhibited by aspirin and other NSAIDs (Vane, 1971). The inhibition by aspirin is due to the irreversible acetylation of the COX active site of these enzymes, while leaving the peroxidase activity of the enzyme unaffected. In contrast, other NSAIDs such as ibruprofen or indomethacin produce reversible or irreversible inhibition by competing with the substrate AA for the active site of the enzyme. The crystal structures of COX isoenzymes were solved recently. Although the overall topology of these enzymes is similar, the active of COX-2 enzyme was larger and more accommodating than that of COX-1 (Picot *et al.*, 1994; Kurumbail *et al.*, 1996).

The primary structures of COX-1 and COX-2 proteins from numerous species are known that both isoforms have a molecular weight of about 71 Kda and contain single peptides of varying lengths (Vane *et al.*, 1998). The mature form of COX-1 contains 576 amino acids while that of COX-2 contains 587 amino acids. There is a 60% to 65% sequence identity between COX-1 and COX-2 from the same species and 85% to 90% identity among individual isoforms from different species.

The majority of sequence differences between both isoforms occur in the membrane binding domains (Otto and Smith, 1996; Spencer *et al.*, 1999). A unique difference between COX-1 and COX-2 is 18 amino acids inserted 6 residues in from the C terminus of COX-2 that are not present in COX-1.

In additional, COX isoforms also separate at the cellular level since COX-1 is found attached only to the membrane of the endoplasmic reticulum (ER), whereas COX-2 is located on the nuclear membrane as well as on the ER (Regier *et al.*, 1993; Otto and Smith, 1994; Morita *et al.*, 1995). The reason for this selective localization of the isoforms may lie in the different sequence of the C terminus.

In *COX* gene structure, *COX-1* and *COX*-2 gene map to chromosome regions 9q32-q33.3 and 1q25.2-q25.3, respectively (Vane *et al.*, 1998) and exhibit only 61% homology (Hla and Neilson, 1992). The human *COX-2* gene at 8.3 Kb is a small immediate early gene, while human

COX-1 originates from a much larger gene which is 22 Kb. The gene products also differ, with the mRNA for the COX-2 enzyme being approximately 4.5 Kb and that of COX-1 enzyme being 2.8 Kb (Otto and Smith, 1995; Herschman, 1996). In addition, its expression pattern is markedly different. The constitutive isoform, COX-1, is constitutive expression in nearly all cell types at a constant level, whereas the induced isoform, COX-2, is normally absent from cells and when induced resulting as the protein levels increase and in a matter of hours after a single stimulus (Otto and Smith, 1995; Herschman, 1996).

8. Physiological and pathological functions of COX-1 and COX-2

The main reason for classifying COX-1 and COX-2 as physiological and pathological functions is that most of the stimuli known to induce COX-2 are those related with inflammation, i.e., bacterial lipopolysaccharide (LPS) and cytokines such as interleukin (IL)-1, IL-2, and tumor necrosis factor (TNF)-α. Furthermore, the corticosteroids and the anti-inflammatory cytokines, including IL-4, IL-10, and IL-13, have been shown to decrease the induction of COX-2 (Otto and Smith, 1995; Bakhle and Botting, 1996; Onoe *et al.*, 1996). The physiological roles of COX-1 have been inferred from the suffering side effects of NSAIDs, which while inhibiting PG biosynthesis at inflammatory sites also inhibit constitutive biosynthesis. Therefore, COX-1 produces PGs in the stomach and intestinal to maintain the integrity of the mucosal epithelium and its inhibition leads to gastric damage, hemorrhage, and ulceration (Vane *et al.*, 1998). This raises the possibility that attempt to explain the respective roles of COX-1 and COX-2 relating with various biological processes, as described below:

a. Gastrointestinal tract

In most species, including human, the bulk of the cytoprotective PGs in the stomach are synthesized by COX-1. These PGs reduce gastric acid secretion, exert direct vasodilator action on the vessels of the gastric mucosa and stimulate secretion of mucous and bicarbonate which forms a protective barrier (Gustafson-Svard *et al.*, 1996). However, Mizuno *et al.* reported that COX-2 mRNA and protein were involved in the repairing process of ulcer healing and that specific antagonists of COX-2 delayed healing in mice (Mizuno *et al.*, 1997) and rat (Schmassman *et al.*, 1998). Interestingly, it has been reported that COX-2 was highly expressed in human and animal colon cancer as well as in human gastric or colorectal adenocarcinoma

Table 1. The staging guidelines of the stomach cancer (Fleming *et al.*, 1997).

Primary Tumor (T)

TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
TIS Carcinoma in situ: intraepithelial tumor without invasion of lamina propria
T1 Tumor invades lamina propria or submucosa
T2 Tumor invades muscularis propria or subserosa*
T3 Tumor penetrates serosa (visceral peritoneum) without invasion of adjacent structures**' ***
T4 Tumor invades adjacent structures**' ***

Regional Lymph nodes (N)

NX Regional lymph node(s) cannot be assessed
N0 No regional lymph node metastasis
N1 Metastasis in 1 to 6 regional lymph nodes
N2 Metastasis in 7 to 15 regional lymph nodes
N3 Metastasis in more than 15 regional lymph nodes

Distant Metastasis (M)

MX Distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis

Stage grouping

Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T1	N1	M0
	T2	N0	M0
Stage II	T1	N2	M0
	T2	N1	M0
	T3	N0	M0
Stage IIIA	T2	N2	M0
	T3	N1	M0
	T4	N0	M0
Stage IIIB	T3	N2	M0
Stage IV	T4	N1	M0
	T1	N3	M0
	T2	N3	M0
	T3	N3	M0
	T4	N2	M0
	T4	N3	M0
	Any T	Any N	M1

*Note: A tumor may penetrate the muscularis propria with extension into the gastrocolic or gastrohepatic ligaments, or into the greater or lesser omentum without perforation of the visceral peritoneum covering these structures. In this case, the tumor is classified T2. If there is perforation of the visceral peritoneum covering the gastric ligaments or the omentum, the tumor should be classified T3.

**Note: The adjacent structures of the stomach include the spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, and retroperitoneum.

***Note: Intramural extension to the duodenum or esophagus is classified by the depth of greatest invasion in any of these sites, including stomach.

Table 2. The comparison of staging systems for colorectal cancer.

Primary Tumor (T)

TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
TIS Carcinoma in situ: intraepithelial tumor or invasion of lamina propria*
T1 Tumor invades or submucosa
T2 Tumor invades muscularis propria
T3 Tumor invades through the muscularis propria into the subserosa, or into nonperitonealized pericolic or perirectal tissues
T4 Tumor directly invades other organs or structures, and/or perforates visceral peritoneum**

Regional Lymph nodes (N)

NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Metastasis in 1 to 3 regional lymph nodes
N2 Metastasis in 4 or more regional lymph nodes

Distant Metastasis (M)

MX Distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis

Stage grouping

AJCC/UICC				**Dukes'**
Stage 0	Tis	N0	M0	A
Stage I	T1	N0	M0	A
	T2	N0	M0	A
Stage II	T3	N0	M0	B
	T4	N0	M0	B
Stage III	Any T	N1	M0	C
	Any T	N2	M0	C
Stage IV	Any T	Any N	M1	D

*Note: Tis includes cancer cells confined within the glandular basement membrane (intraepithelial) or lamina propria (intramucosal) with no extension through the muscularis mucosae into the submucosa.

**Note: Direct invasion in T4 includes invasion of other segments of the colorectum by way of the serosa; for example, invasion of the sigmoid colon by carcinoma of the cecum.

(The original source for this material is the AJCC® cancer Staging Manual, 5th edition (1997) published by Lippincott-Raven Publishers, Philadelphia, Pennsylvania.)

tissues compared with those in normal tissues, while COX-1 mRNA levels were not elevated in the carcinoma (Gustafan *et al.*, 1996; Tsujii *et al.*, 1997; Ristimaki *et al.*, 1997).

b. The kidney

PGs are important in the maintenance of kidney function; therefore patients are at risk of various disorders when PG synthesis is reduced by chronically administered NSAIDs. Those kidney cells that synthesize PGs contain mostly COX-1, whereas low levels of COX-2 mRNA have also been detected (Harris *et al.*, 1994). COX-2 metabolites are involved in the regulation of renin-angiotensin system and/or glomerular hemodynamics (Harris *et al.*, 1998). Renal blood flow and glomerular filtration rate become progressively dependent upon PG synthesis under conditions of volume depletion or reduced renal perfusion pressure (Kulkarni and Varghese, 1998).

c. The lung

Airway hyperreactivity, a feature of allergic asthma, is related with inflammation of the airways. Samet *et al.* reported that levels of COX-2 mRNA and protein were increased, with no change in COX-1 levels, in the airway smooth muscle cells treated with proinflammatory cytokines (Samet *et al.*, 1996). COX-2 is probably up regulated in the inflamed lungs of the asthmatics resulting in increased production of bronchoconstructor PGs which exert an exaggerated effect on the bronchiolar smooth muscle that has become hyperreactive to constrictor agents.

e. Central nervous system (CNS)

COX-1 is distributed in neurones throughout the brain, but it is predominance in the forebrain, where PGs may be involved in complex integrative functions, such as control of the autonomic nervous stimulation and sensory processing (Yamagata *et al.*, 1993; Breder *et al.*, 1995; Breder and Saper, 1996). COX-2 is also constitutively expressed in only a few organs and one of those is the brain, which this expression is restricted to certain parts of the CNS, notably cortex, hippocampus, hypothalamus, and spinal cord (Breder *et al.*, 1995; Breder and Saper, 1996). The role of COX-2 in various neurodegenerative disorders has been reported, such as, PGE_2 involved in the febrile response originates from COX-2 induced in endothelial cell lining on the cerebral blood vessels (Cao *et al.*, 1996; Pasinetti and Aisen, 1998).

f. Inflammation and cancer

Chronic inflammation is regarded as a risk factor for epithelial carcinogenesis (Weitzman and Gordon, 1990). The inflammation, *per se*, is associated with increased synthesis of PGs partly through cytokine-mediated COX-2 induction. Thus, it can be concluded that a cause-and-effect linkage between chronic inflammation and carcinogenesis may be through the overexpression of COX-2, leading to a reasonable mechanism by which chronic inflammation increase the risk of cancer.

The growth and progression of cancers is suspected to be associated with COX-2 expression. Subsequent experiments appear to support this view. For example, increased amounts of PGs were found in adenomas and colorectal cancers (Tsujii and Dubois, 1995) and this was correlated with increased expression of COX-2, but not COX-1, in 70% to 80% of such cancers (Eberhart *et al.*, 1994; Kargman *et al.*, 1995; Dannenberg and Zakim, 1999). Related data is the decreased intestinal tumorigenesis in mice with inactivation of the *COX-2* gene. As described above, the *APC*-mouse was an animal model of human FAP, and the number of intestinal polyps was reduced by 87% in *COX-2* null mice and by 66% when one copy of the *COX-2* gene was disrupted (Oshima *et al.*, 1996). Taken together, these results suggest that induction of COX-2 is an early event and also plays an important role in the development of the cancers.

9. Regulations of COX-1 and COX-2 expression

9.1 Regulation of COX-1 expression

Although COX-1 is constitutively expressed in most tissues and the expression does not very large in adult animals, thereby it has been difficult to investigate transcriptional regulation of the *COX-1* gene. Nevertheless, COX-1 protein is favorably expressed at high levels in selected cells and tissues, such as endothelium, monocytes, platelets, renal collecting tubules, and seminal vesicles. These studies also indicate that the levels of *COX-1* gene expression are highly regulated (Smith *et al.*, 2000). One of these cells which express constitutive high levels of COX-1, endothelial cells, have been used with moderate success to determine transcriptional regulation of this gene (DeWitt *et al.*, 1983). Interestingly, the *COX-1* gene lacks a TATA box that contains multiple start sites for transcription (Kraemer *et al.*, 1992; Xu et *al.*, 1997). There are mainly two elements, contribute to constitutive expression of COX-1 in human umbilical vein endothelial

cells (HUVEC), have been identified (Xu *et al.*, 1997). The results of gel shift assay have shown that the Sp1 *cis*-regulatory elements in the human COX-1 promoter, at position –111/-105 and –610/-604, were bound by the *trans*-activating Sp1 protein. The deletion of either site resulted in a reduction of approximately 50% in basal transcription, and deletion of both sites lead to a reduction of approximately 75% (Smith *et al.*, 2000). Hence these Sp1 sites are the only *cis*-acting elements documented to regulate *COX-1* transcription.

9.2 Regulation of COX-2 expression

Increased amounts of COX-2 were found commonly in both precancerous and cancerous tissues (Dannenberg *et al.*, 2001). Overexpression of COX-2 seems to be a consequence of both increased transcription and enhanced mRNA stability (Shao *et al.*, 2000; Dixon *et al.*, 2000). Several inducible enhancers including oncogenes, growth factors, cytokines, chemotherapy, and tumor promoters have been shown to stimulate *COX-2* transcription through protein kinase C (PKC) and RAS-mediated signaling (Fig. 4) (Subbaramaiah and Dennenberg, 2003). For instance, COX-2 level was overexpression in HER-2/neu-positive breast cancer as result of elevated RAS signaling (Subbaramaiah *et al.*, 2002). Microtubule-interfering agent like taxane has also been shown to induce COX-2 expression via activating PKC and mitogen-activated protein kinase (MAPKs) (Fig. 4) (Zhang *et al.*, 1998). Numerous transcription factors including activator protein 1 (AP-1), nuclear factor interleukin-6 (NF-IL6), nuclear factor kB (NF-kB), and polyomavirus enhancer activator 3 (PEA3) can modulate the transcription of *COX-2* (Smith *et al.*, 2000; Subbaramaiah *et al.*, 2002). Additionally, the histone acetyltransferase activity of the cAMP regulatory binding protein (CREB)/p300 co activator complex was found to be important for AP-1-mediated induction of COX-2 (Subbaramaiah *et al.*, 2002). Although many factors enhance the transcription of *COX-2*, much less is known about negative modulators. Wild-type *p*53, but not mutant, markedly suppresses *COX-2* transcription by competing with TATA-binding protein (TBP) for binding to the TATA box (Subbaramaiah *et al.*, 1999).

Interestingly, there are several studies reported that COX-2 levels were higher in certain cancers that express mutant rather than wild-type *p*53 (Leung *et al.*, 2001; Ristimaki *et al.*, 2002). Therefore, these results suggested that the balance between oncogene activation and tumor suppressor gene inactivation affect the expression of COX-2.

In post-transcriptional mechanism of COX-2, the *COX-2* mRNA contains a large 3'-uptranslated region (UTR) that contains a series of Shaw-Kamen sequences, AUUUA or AU enriched elements (ARE), conferring message instability (Appleby *et al.*, 1994). The enhancers induce COX-2 by enhancing mRNA stability leading to an increase of expression (Sheng *et al.*, 2000; Zhang *et al.*, 2000). Additionally, extended binding of RNA binding protein, HuR, to the AU-enriched elements of COX-2 is responsible at least in part, for the remarked increase in message stability in colon cancer cells (Fig. 4) (Dixon *et al.*, 2001). Furthermore, activation of extracellular signal regulated kinase 1/2 (ERK1/2) and p38 stabilize *COX-2* mRNA in addition to stimulation of transcription (Subbaramaiah and Dannenberg, 2003).

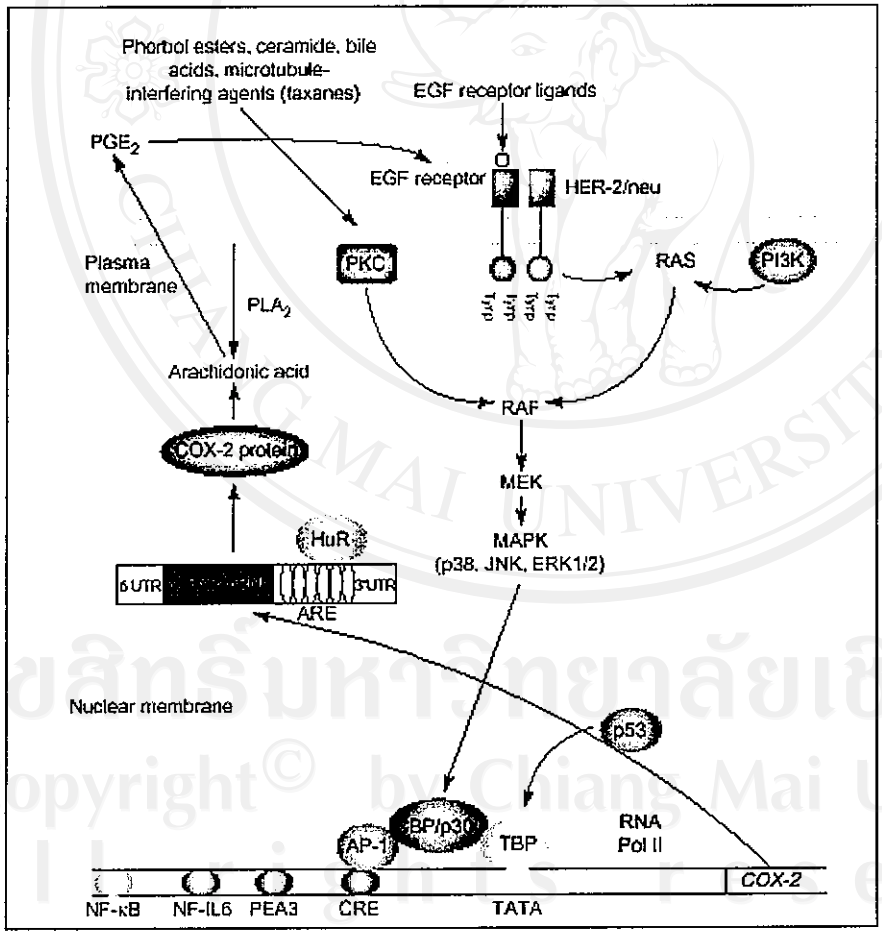

Figure 4. Regulation of COX-2 in cancer.

(Modified from Subbaramaiah K, Dannenberg AJ. Cyclooxygenase-2: a molecular target for cancer prevention and treatment. TRENDs Pharmacol Sci 2003; 24(2): 96-102.)

10. Expression of COX-2 in stomach and colorectal cancer

The relation levels of COX-2 and COX-1 mRNA and/or protein expression in stomach and colorectal cancer have been evaluated by a number of different groups which have mostly reported increased levels of COX-2 expression in these carcinomas. For example, Eberhart *et al.* determined *COX* mRNA level by Northern blot analysis from human colorectal cancers specimens including 14 carcinomas, 6 adenomas, and 14 polyps. In each case, accompanying normal mucosa was collected for comparison, but without paired normal mucosa in polyps resection. It was found that *COX-2* mRNA, but not COX-1, overexpressed in 86% of carcinomas, compared with accompanying normal mucosa, and up-regulated in 43% of polyps (Eberhart *et al.*, 1994). In addition, Fujita *et al.* have augmentative studied the relationship between the *COX-2* mRNA levels and pathological characteristics (TNM classification) in 43 colorectal carcinomas. This study appeared that COX-2 levels were significantly higher in tumors with larger size and in those with deeper invasion, but were not correlated with metastasis. This suggests that larger carcinomas produce more COX-2 to supply their own growth (Fujita *et al.*, 1998).

In detection of COX-2 protein expression, Sano *et al.* demonstrated the expression and localization of COX protein in 15 subjects with diagnosed colorectal cancer by immunohistochemistry, which includes steps of tissue-slide preparation, staining with labeled specific COX-1 or COX-2 antibodies, and microscopic scoring. Normally, the extent and intensity of the staining is graded on a scale, such as 0 to 4+, by a blinded observer on two separate occasions using coded slides, and an average score is calculated further. This study showed that the levels of COX-2 protein expression were much greater than that of the other cell types, whereas the COX-1 expression was weak in both normal and cancerous tissues (Sano *et al.*, 1995). Western blot analysis is also a widely used method for the identification and quantitation of COX protein expression. For instance, Cianchi *et al.* determined the expression of COX-2 protein in 31 colorectal adenocarcinomas. In this investigation, Western blotting was used to examine and confirm the COX-2 protein overexpression that had been detected in 15 colorectal cancer specimens by immunohistochemistry. The results showed higher amounts of COX-2 in the neoplastic tissue of 12 specimens, whereas the expression of COX-1 was decreased in 12 out of the 15 cancerous specimens comparing with paired normal mucosa (Cianchi *et al.*, 2001). The same study demonstrated that COX-2 was up regulated in colorectal cancers relative to normal

mucosa, but COX-1 was detected at an equivalent level in tumors and normal tissues (Molina *et al.*, 1999).

For the investigations of COX-1 and COX-2 expression in stomach cancer, the expression was detected both at mRNA and protein levels by Northern blot analysis and immunohistochemistry or Western blot analysis, as well as detections of the COX expression in colorectal cancer. Several studies have demonstrated increase levels of COX-2 mRNA and/or protein expression, but not COX-1 mRNA and/or protein, in stomach cancer compared with paired normal mucosa (Ristmaki *et al.*, 1997; Uefuji *et al.*, 1998; Murata *et al.*, 1999). Additionally, COX-2 was up regulated in precancerous lesions (metaplastic and adenomatous cells) of the stomach, suggesting an early role of COX-2 in gastric cancer development (Lim *et al.*, 2000). Moreover, the overexpression of COX-2 was also associated with more advanced tumor stage of the stomach and often with lymph node metastasis (Yamamoto *et al.*, 1999).

While COX-2 protein was overexpressed, COX-1 protein have been found to be similar or reduced in cancerous tissues in comparison to normal mucosae. As states above, there are still no consensuses of the role of COX-1 in tumorigenesis. In additional, although overexpression of COX-2 was repeatedly reported in human cancer, none of them was investigated in Thai cancer patients. Thus, this study aimed to investigate the expression levels of COX protein, particularly COX-2, in Thai patients with stomach cancer and colorectal cancer by Western blot analysis. Furthermore, the relationship between expression of COX-2 and COX-1, and the pathological features were investigated in order to the roles of COX enzyme in tumor progression.