I. INTRODUCTION

Cancer is a genetic disorder (Karp, 1999) that is caused by alterations of the important growth-controlling genes. These genetic alterations caused cancer cell to proliferate uncontrollably and transmit the genetic defect to daughter cells. It is generally known that these mutations do not altogether occur in one step but they are a multistep process that starts with slight genetic alterations continue to stepwise accumulation of multiple genetic defects. Finally, a normal cell transforms into a cancer cell that has a completed malignant phenotype.

At present, cancer is a crucial public health problem of the world. Worldwide about 6.3 million people die from cancers every year, which is the third most common cause of death. About 765,000 and 525,000 people die from gastric and colorectal cancer every year, which make them the second and third most common cause of cancer death worldwide after lung cancer, respectively (Goldinger, 2001). In this study, we focused on gastrointestinal tract cancers including large bowel and stomach.

Colorectal cancer is a major form of cancer in the Western world. In the United States, colorectal cancer is the most important causes of cancer deaths after lung cancer. It has been estimated that 50% of those over 70 years old have colorectal adenoma and 10% of those will develop to adenocarcinoma (Kinzler and Vogelstein, 1996). In gastric cancer, there is a great variation in the geographic distribution of this cancer. The highest incidences were found in Japan and Costa Rica (Correa, 1992). Although the incidence of gastric cancer has been diminishing in Western countries, it is still the leading cause of cancer death worldwide (Alexander *et al.*, 1997). In Thailand, according to the cancer incidence reported from the National Cancer Institute, Department of Medical Services, Ministry of Public Health for Thai patients with cancers in 1993 indicated that stomach and colorectal cancer are the third and sixth most common cancer in males and the fifth and ninth in females, respectively; which account for 9.8% of all cancers.

The reduction of risk of developing colorectal and gastric cancer in chronic users of nonsteroidal anti-inflammatory drugs (NSAIDs) was initially reported by studies performed in Finland and Sweden on patients with rheumatoid arthritis (Isomaki *et al.*, 1978; Laakso *et al.*,

1986; Gridley *et al.*, 1993). Since these patients used aspirin or other NSAIDs in high dose for prolonged periods of time, it is possible that these drugs were responsible for the reduction of the cancer incidence. In fact, both observational and controlled human studies have shown that NSAIDs, especially sulindac, cause regression of colorectal adenomatous polyps in patients with familial adenomatous polyposis (FAP), which is an inherited condition leading to colorectal cancer (Giardiello, 1994). Additionally, several epidemiological studies have shown that prolonged use of aspirin is associated with reduced risk of colorectal cancer by 40-50% (Thun, 1994; Giardiella *et al.*, 1995; Giovannucci *et al.*, 1995). Moreover, in a large prospective mortality study, the use of aspirin was associated with reduced risk of esophagus, gastric and colorectal cancers, but not in cancer outside gastrointestinal tract (Thun *et al.*, 1993).

Hence, it seems remarkable that NSAIDs may be chemoprotective against colorectal and gastric cancer, but the precise mechanisms by which NSAIDs exert their chemopreventive effects are not fully understood. In addition, NSAIDs are the best known to inhibit the cyclooxygenase (COX) enzyme, which may account for their anti-cancer activity; therefore, the investigation of the role of COX in colorectal and gastric carcinogenesis has become a new area of cancer researches.

COX or prostaglandins H_2 synthase (PGHS) is the enzyme that catalyzes the biosynthesis of the prostaglandins (PGs) from arachidonic acid (AA) (Vane *et al.*, 1998). The initial step is the oxidation of AA via cyclooxygenase activity to the hydroperoxy endoperoxide PGG_2, which is subsequently reduced to the hydroxy endoperoxide PGH_2 via its peroxidase activity. The PGH_2 is transformed by specific synthases into the primary prostanoids, i.e., PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (prostacyclin) and TxA_2 (thromboxane A_2). So far, two enzyme isoforms have been identified, named as cyclooxygenase-1 (COX-1) and COX-2 (Yokoyama and Tanabe, 1989; Hla and Neilson, 1992).

COX-1 is constitutively expressed and prostaglandins synthesized by the COX-1 pathway are thought to be responsible for cytoprotection of the stomach (Robert, 1983), regulation of renal blood flow (Whelton, 1999) and production of a proaggregatory prostanoid, TxA_2, by the platlets (Funk *et al.*, 1991). In contrast, COX-2 is not constitutively expressed in appreciable amounts by most normal tissue, but its expression is rapidly induced by certain

inflammatory cytokines, tumor promoters, growth factors and oncogenes (Prescott and Fitzpatrick, 2000).

Several studies have shown that the levels of mRNA and protein of COX-2, but not COX-1, are elevated in colorectal and gastric cancers compared with the adjacent normal mucosa (Eberhart et *al.*, 1994; Ristimaki *et al.*, 1997; Vefuji *et al.*, 1998; Cianchi *et al.*, 2001). One of the most important studies showing the role of COX-2 in colorectal carcinogenesis was the determination of the effects of COX-2 gene knockout on intestinal polyposis development using adenomatous polyposis coli ($Apc^{\Delta 716}$) gene knockout mice, a mouse model of human FAP. The *Apc* gene is a tumor suppressor gene and inactivation of this gene leads to the development of multiple adenomatous polyps, which can progress into colorectal cancer. This study transferred a knockout mutation of the COX-2 gene (*Ptgs* 2) into the $Apc^{\Delta 716}$ knockout mice by two successive crosses and generated double-mutant mice that carried $Apc^{\Delta 716}$ (+/-) *Ptgs* 2 (+/-), $Apc^{\Delta 716}$ (+/-) *Ptgs* 2 (-/-) and $Apc^{\Delta 716}$ (+/-) *Ptgs* 2 (+/+) mutations. The last genotypes were used as positive controls. It was found that a *Ptgs* 2 null mutation decreased the number and size of the intestinal polyps dramatically, indicating that COX-2 plays a key role in tumor formation (Oshima *et al.*, 1996).

Nevertheless, COX-2 also plays an important role in the tumor progression. The one of the previous study has shown that levels of COX-2 mRNA were significantly higher in tumors with larger sizes and those with deeper invasions in comparison to normal tissues (Fujita *et al.*, 1998). This results suggested that the COX-2 expression level increases significantly upon progression of adenomas to carcinomas. In addition, COX-2 affects many processes that are important in carcinogenesis; for instance, COX-2–generated prostaglandins have been demonstrated to be immunosuppressive that help the tumor cells to escape from immunologic surveillance (Williams *et al.*, 1999) and stimulate cell proliferation (Sheng *et al.*, 1998). The overexpression of COX-2 led to alterations in the phenotype of intestinal epithelial cells involving an increase in cell matrix adhesion and inhibition of apoptosis that could enhance their tumorigenic potential (Tsujii *et al.*, 1997).

Moreover, COX-2 may contribute to tumor angiogenesis due to several reasons including: (1) COX-2 increased expression of vascular endothelial growth factor (VEGF), (2) the ecosanoid products from COX-2, i.e., TxA_2, PGE_2, and PGI_2 can directly stimulate endothelial

cell migration and growth factor-induced angiogenesis and (3) COX-2 inhibited endothelial cell apoptosis by stimulation of Bcl-2 or Akt activation (Gately, 2000).

All of the above, it is clear that COX-2 plays an important role in tumor formation and progression. Although several groups have studied the COX-2 expression in colorectal and gastric cancer, but to our knowledge none of them were carried out in Thai patients. In addition, although these cancers are often found in Thai population, they have not been extensively studied. Moreover, knowing the incidence of COX-2 overexpression in Thai patients may provide primary information whether COX-2 inhibition will be useful for Thai population.

In the present study, the expression levels of COX-2 protein in Thai patients with stomach and colorectal cancer were examined together with the corresponding normal tissue. Furthermore the relationships between COX-2 expression and the conventional pathological features, including tumor staging, histological grading, and clinical outcomes, were investigated in order to elucidate the role of COX-2 in the tumor formation and progression.

Aims of the study

To study the incidence of COX-2 overexpression in Thai patients with colorectal and stomach cancers and the relationship between expression of COX-2 protein and pathological features including tumor staging, histological grading, and clinical outcomes.