DISCUSSION

Stomach and colorectal cancers are the most common causes of cancer death in the world. Although a cause of the cancers is not clearly known, many researchers have been trying to elucidate the molecular events leading to cancer development and progression. Originally, several epidemiological studies have suggested the NSAIDs reduce the incidence of and mortality from colorectal and gastric cancers (Rosenberg *et al.*, 1991; Thun *et al.*, 1993; Husain *et al.*, 2002). In addition, NSAIDs can reduce the formation of adenomatous polyps in patients with familial adenomatous polyposis (Giardiello *et al.*, 1993; Thun *et al.*, 1993) and in Apc Min mouse (Jacoby *et al.*, 1996). However, the exact mechanisms of NSAIDs on cancer prevention have not been clarified. One of the possible roles of NSAIDs is via the inhibition of COX enzymatic activity leading to chemo-preventative effect.

COX exists in two isoforms, which include COX-1 and COX-2. COX-1 is the constitutive enzyme that produces the prostaglandin (PG) for maintaining the physiological functions such as cytoprotection of the gastric mucosa, renal function, and vascular homeostasis. Several studies have shown that the COX-1 levels are maintained at constant levels in the most tissues (Smith and Bell, 1978; Dewitt *et al.*, 1983; Simmons *et al.*, 1991; Kujubu *et al.*, 1993). COX-2 is an inducible enzyme that produces PGs involving inflammation and growth (Dewitt and Smith; 1988; Funketal *et al.*, 1991; Kujubu *et al.*, 1991; Hla and Neilson, 1992; O'Banion *et al.*, 1992). The expression of COX-2 in various cell types is increased ten- to eight-fold by stimulations of growth factors, phorbol ester and IL-1 (Kujubu *et al.*, 1991; Crofford *et al.*, 1994; Hempel *et al.*, 1994).

There have been many studies on the levels of COX-2 protein expression in gastrointestinal tract cancers using Western blot analysis and have shown enhanced expression of COX-2 in both colorectal and gastric tissues as compared with normal tissues such as overexpressed COX-2 levels in 10 of 15 (66.7%) (Murata *et al.*, 1999), 73 of 104 (70.2%) (Lim *et al.*, 2000) and 38 of 50 (76%) (Husian *et al.*, 2003) of gastric carcinomas, and in 19 of 25 (75%) (Kargman *et al.*, 1995) and 12 of 15 (80%) (Cianchi *et al.*, 2001) of colorectal carcinomas.

These results suggest that COX-2 may play an important role in tumorigenesis of the stomach and large bowel. The present study demonstrated that overexpression of COX-2 protein was presented in only 13 out of 44 (29.5%) colorectal tumor tissues and in 1 of 20 (5%) gastric tumors from Thai patients and none of the adjacent normal tissues was found to possess a detectable level of COX-2. The low incidence of COX-2 overexpressed in gastric and colorectal cancer in Thai population, lead to the question whether COX-2 inhibitor will be useful as a anti-cancer drug in Thai population, since it has been demonstrated that tumor growth and angiogenesis could be suppressed by selective COX-2 inhibitor only if the tumor cells expressed COX-2 (Sawaoka *et al.*, 1999).

It has been reported the relationship between COX-2 levels and pathological features of colorectal tumor,. i.e., larger sizes and deeper invasion, but was not correlated with whether the patients had distant metastasis or not (Fujita *et al.*, 1998). In addition, COX-2 expression increased in a size-dependent manner in gastric hyperplastic polyp, precancerous stage, significantly (Kawada *et al.*, 2003). In this study, COX-2 overexpression in both cancers was not correlated with tumor sizes and the depth of tumor invasion, however the case with COX-2 expression was found in the tumor tissues with large size and deep invasion (advanced tumor). These results, taken together, support a hypothesis that the COX-2 level increases significantly during progression of adenomas to carcinomas. Although this hypothesis is based on the supposition that the adenomas in familial adenomatous polyposis are of the same nature as the precursors of sporadic colorectal carcinomas, it appears reasonable because suppressive effects of NSAIDs have been reported in the colorectal adenomas as well (Nakajima *et al.*, 1997; Sendler *et al.*, 1998).

Using immunohistochemistry, Ristimaki *et al* reported that COX-2 immunoreactivity was found in the cytoplasm of human gastric carcinoma cells and some hyperplastic glands, but not in the surrounding stroma and glands of normal morphology in the human stomach (Ristimaki *et al.*, 1997). Additionally, there has been previously reported that gastric carcinoma cell lines expression different levels of COX-2 such as; MKN28 cells (well differentiated adenocarcinoma) expressed COX-2 weakly, but MKN45 cells (poorly differentiated adenocarcinoma) express strongly (Tsuji *et al.*, 1996). Interestingly, COX-2 overexpression in colorectal tumors was found to be correlated with histological differentiation ($P<0.05$) in this study, which indicated the poor

prognostic implication of COX-2 expression. However, histological differentiation was not correlated with COX-2 overexpression in gastric tumor, which may results from the limited number of patients studied.

A previous study have shown that when colon cancer cell line (CaCo-2) was permanently transfected with COX-2 expression vector, it acquired an increased invasiveness and capable of activating membrane-type metalloproteinase and metalloproteinase-2, suggesting that COX-2 may induce the metastatic potential as well as carcinogenicity (Tsuji *et al.*, 1997). In lung adenocarcinomas, markedly higher level of COX-2 expression was found in lymph node metastasis tumors more frequently than that in the primary tumors (Hida *et al.*, 1998). Furthermore, COX-2 overexpression was also correlated with the invasion to lymphatic vessels in the gastric wall and with the metastasis to the lymph nodes of gastric tumors (Murata *et al.*, 1999). However, in this study it was found that although COX-2 was significantly overexpression in colorectal tumor tissues compared with the corresponding normal colorectal tissues and was correlated with histological changes, it was not significantly correlated with many pathological characteristics, i.e., lymphatic invasion, venous invasion, perineural invasion, distant metastasis, lymph node metastasis, and TNM stage grouping (early and late stage). Interestingly, overexpression of COX-2 was found more frequently in tumors with lymph node metastasis and later stage of tumors (stage III and IV), although it was not statistically significant. This may due to the fact that there was a small number of tumors in Thai population exhibit COX-2 overexpression (only 29.5%). Although larger group of cancer patients needed to be studied, this observation implies that the rate of overexpression of COX-2 may be partly dependent the race and genetic backgrounds of the patients.

The majority of previous studies have reported that COX-1 protein levels were quite similar between in tumor tissues and normal tissues (Molina *et al.*, 1999; Murata *et al.*, 1999). However some studies have noted that COX-1 level can be either reduced or increased in tumor tissues (Murata *et al.*, 1999; Cianchi *et al.*, 2001). Therefore, there is still no clear consensus about the role of COX-1 in tumorigenesis. The present study demonstrated that the levels of COX-1 protein in tumor tissues were varied either reduced, increased or unchanged expression in comparison to normal tissues. However, the majority of colorectal (47.8%) and gastric (80%) tumors tended to possess a decreased level of COX-1 protein compared to normal tissue.

Interestingly, 10 of 44 tumor tissues (22.7%) showed an increased level of COX-1 protein, indicating that COX-1 may also play an important role in promoting and maintaining the neoplastic state as well as COX-2.

COX-1 expression was considered to be constitutive and generated prostaglandin for normal physiological function. However, a number of studies have recently shown that COX-1 expression can be induced in vitro by tobacco carcinogen (Rioux *et al.*, 2000), VEGF (Bryant *et al.*, 1998)), arachidonic acid and PGE2 (Maldve *et al.*, 2000). In addition, an elevated level of COX-1 expression has been reported in mouse lung tumors (Bauer *et al.*, 2000), human breast cancer (Hwang *et al.*, 1998), human ovarian cancer (Gupta *et al.*, 2003) and prostrate carcinoma (Kirschenbaum *et al.*, 2000). This leads the question whether it is worthy to try to develop a selective COX-2 inhibitor for the purpose of using them as an anti-cancer drug.

A study performed by Sales and his group has recently demonstrated that COX-1 may regulate COX-2 expression through its enzyme product. Overexpression of COX-1 in Hela cells resulted in overexpression of COX-2 and concomitant with increased PGE2 synthesis. Treatment of Hela cells overexpressing COX-1 with dual COX inhibitor indomethacin or selective COX-2 inhibitor NS-398 significantly reduced PGE2 synthesis. Indomethacin, but not NS-398 treatment abolished the up-regulation of induction COX-2 and PGE2 in Hela cells, suggesting that the observed up-regulation of COX-2 and PGE2 synthesis was mediated by COX-1 enzyme product (Sales *et al.*, 2002). From their results, the authors proposed that COX-1 may act in autocrine/paracrine fashion to regulate COX-2 expression.

It is possible that expression of the two isoforms of COX is regulated by each other. During the early stage of tumorigenesis, a small increase of COX-2 expression may be compensated by the reduction of COX-1 expression as cells try to maintain the total enzyme activity with in the limited range. However, once the tumor has progressed, this balance may be broken. Therefore, at later stage of cancer, tumor cells possess an increase level of COX-1 or COX-2 as either of them can promote and maintain tumor growth. More than 50% of tumor tissues investigated in this study exhibited a decreased level of COX-1, with a small proportion of colorectal tumors (23%) appeared to overexpress COX-1. If our hypothesis is true, cancers that overexpressed COX-1 should not overexpressed COX-2 and *vice versa.* Of 10 colorectal tumors that exhibited an increased level of COX-1, only 3 tumors had overexpressed level of COX-2. On

the other hand, of 13 tumors overexpressing COX-2, only 3 tumor were found to overexpress COX-1. However, the only drawback of this hypothesis is that no significant relationship between overexpression of COX-1 and any of the pathological features was found in this study.

This study has demonstrated that COX-2 was overexpressed in the colorectal tumor tissues from Thai patients and their presence was significantly correlated with poor differentiation of the cancer cell. In addition, overexpression of COX-2 was found more frequently the tumors with larger size, lymph node metastasis, and lymphatic invasion of colorectal and stomach cancers suggesting that COX-2 may be involved in the development and/or progression of these cancers. Although alteration of COX-1 in tumor tissues was not significantly correlated with any of the pathological features, the results obtained from this study raise the possibility that it may play important roles in tumorigenesis. If that was the case, it is necessary to inhibit both isoforms of COX in order to antagonize tumor growth.