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Abstracts of the 2004th Maruyama Memorial Research Fund Prize Memorial Lecture (1)

Clinical Significance of Prostaglandin E Synthase Expression in Colorectal Cancer

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Introduction

Nonsteroidal Anti-inflammatory Drugs and Colorectal Neoplasm

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce the incidence of carcinogen-induced colon tumors in rodents and are associated with a reduced incidence of gastrointestinal cancer in epidemiological studies. NSAIDs inhibit prostaglandin endoperoxide synthase/cyclooxygenase (COX), the rate-limiting enzyme catalyzing the metabolism of arachidonic acid into prostaglandins (PGs), prostacyclin, and thromboxanes **(Fig. 1)**. There are two isoforms of COX: a constitutively produced COX-1 and an inducible COX-2. Several lines of evidence indicate that the anti-neoplastic effect of NSAIDs is attributable to COX-2 inhibition. Selective COX-2 inhibitors reduce the incidence, multiplicity, and size of colonic carcinomas in the azoxymethane rat model, and their effectiveness against human colorectal neoplasms has been shown in epidemiological studies. Thus, COX-2 has received much attention as a molecular target for colorectal cancer prevention and treatment.

Localization of COX-2 in Colorectal Neoplasms

We have previously examined COX-2 expression in human colorectal neoplasm (adenoma and adenocarcinoma) tissues by immunohistochemical analysis. COX-2 immunoreactivity was weak-to-absent in normal colonic mucosa adjacent to tumor tissue **(Fig. 2A)**, whereas COX-2 reactivity was detected in stromal
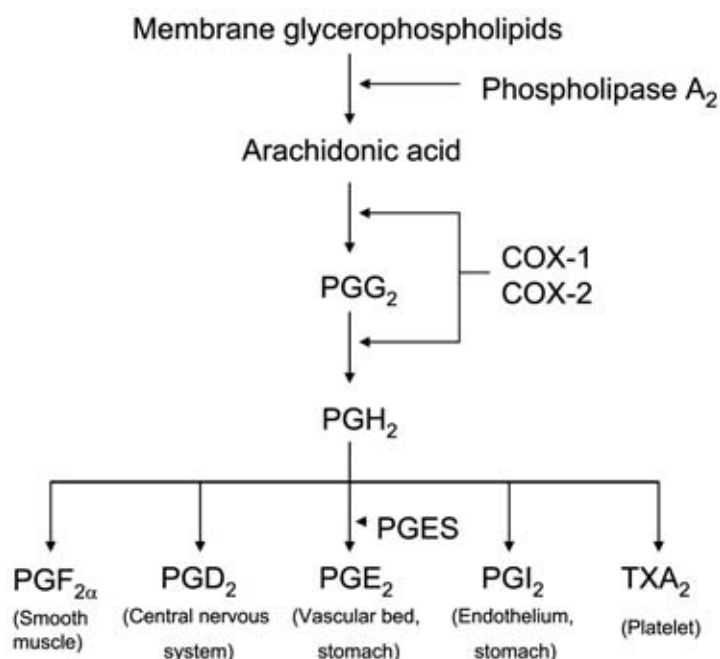
Fig. 1 Arachidonic acid metabolism. The major metabolites of arachidonic acid produced by the cyclooxygenase (COX) pathways are indicated. Examples of tissues in which individual prostanoids exert prominent effects are indicated in parentheses.

cells at the upper border of the lamina propria, just beneath the epithelium of colonic adenomas with mild atypia (**Fig. 2B**). COX-2 reactivity was focally observed in the epithelium of adenoma with severe atypia (**Fig. 2 C**). In cancer tissues, COX-2 immunoreactivity was detected predominantly in epithelial cells (**Fig. 2D**) in 90% of cases. We then examined the relationship between COX-2 expression and clinicopathological factors, including prognosis. Epithelial COX-2 reactivity correlated with the grade of atypia and the size of polyps in colorectal adenoma tissues, whereas it did not show any significant correlation with most clinicopathological factors in patients with adenocarcinoma. In particular, no statistical association was found between COX-2 expression and overall survival or disease-free survival for any patients. Our results have been confirmed by a recent large scale cohort study of 747 patients with colorectal cancer, in which immunohistochemical analysis showed that COX-2 expression is unrelated to overall survival or to disease-free survival. These results suggest that COX-2 expression is an early event in the development of colorectal neoplasms. However, other investigators have reported a positive correlation between COX-2 expression and pathological stage or prognosis. Thus, the significance of COX-2 in colorectal tumorigenesis remains unclear.

PGE Synthase

PGE2 is the most common prostanoid. It was a variety of biological activities and plays essential roles in tumor progression, such as tumor cell proliferation, invasion, angiogenesis, and immunosuppression. PGE2 is produced via three sequential enzymatic reactions: release of arachidonic acid (AA) from membrane glycerophospholipids by phospholipase A2, conversion of AA to the unstable intermediate prostanoid PGH2 by COX, and isomerization of PGH2 to PGE2 by organ-specific PG synthase. In 1999, Jakobsson *et al.* reported that recombinant human microsomal GST-1-like 1 (MGST1-L1), a member of the membrane-associated proteins involved in eicosanoid and glutathione metabolism superfamily that had been listed in nucleic acid data bases, could catalyze the conversion of PGH2 to PGE2 with strict substrate specificity. MGST1-L1 was renamed
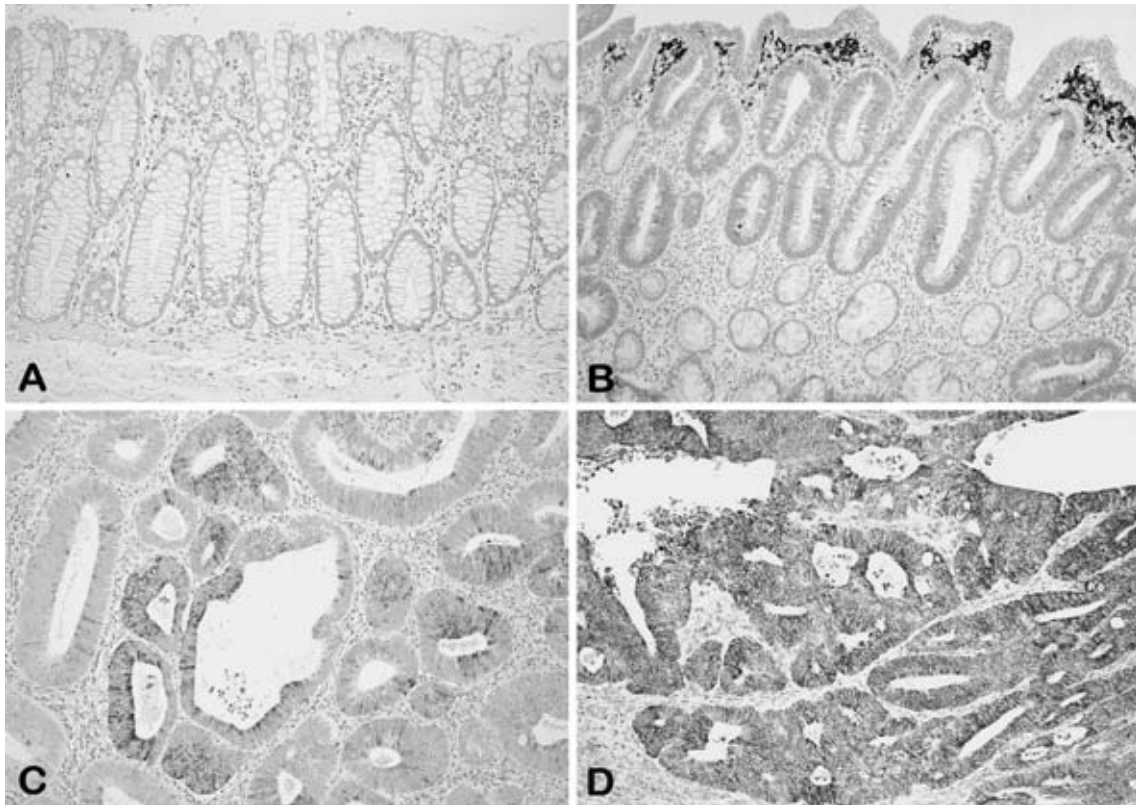
Fig. 2 Immunohistochemical localization of COX-2 in colorectal neoplasm. **A**. Normal colonic mucosa. **B**. Colonic adenomas with mild atypia. **C**. Adenoma with severe atypia. **D**. Well differentiated adenocarcinoma. Original magnification ×20.
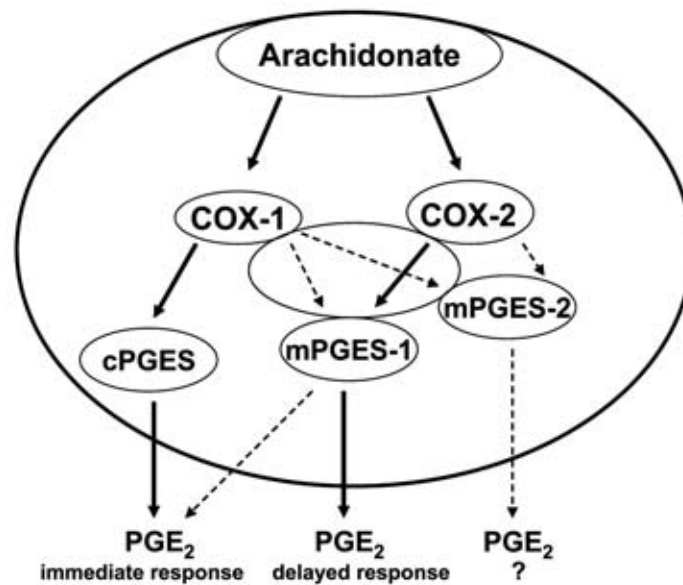

Fig. 3 A schematic model for functional coupling between the two COXs and three PGESs.

microsomal PGES (mPGES). At least three distinct isoforms of PGES have been cloned: one cytosolic PGES (cPGES) and two microsomal fractions, mPGES-1 and mPGES-2. Studies of cells over-expressing these enzymes

have shown that cPGES is constitutively expressed and is functionally coupled to COX-1 in marked preference to COX-2; whereas mPGES-1 is inducible and preferentially coupled to COX-2, causing a delayed PGE2 release response. mPGES-2 has yet to be well characterized, although it has been shown to be glutathione independent, unlike its sister PGES's **(Fig. 3)**. Although expression of these PGESs has been reported in colorectal cancer (CRC), their clinical significance remains unclear. This study aimed to investigate the expression of each PGES in CRC tissue, and to examine the relationship of their expression to various clinicopathological factors and to patient prognosis.

Materials and Methods

A total of 99 patients with CRC who had undergone surgery were examined. mPGES-1 mRNA and protein expression levels were examined with real-time polymerase chain reaction (PCR) and Western blot analysis, respectively. mPGES-1 localization was analyzed immunohistochemically. Immunostaining results for mPGES-1 were compared with clinicopathological factors.

Results

Real-time PCR and Western blot analysis of mPGES-1 mRNA and protein expression showed significantly higher expression levels in CRC tissues than in paired normal tissues. Immunoreactivity for mPGES-1 was predominantly observed in the cytoplasm of the cancer cells in 43 cases (43%). A few stromal cells, such as fibroblasts and macrophages, were weakly positive for mPGES-1. mPGES-1 expression correlated with venous invasion (P=0.02), but not with any other clinicopathological factors. Survival rates were significantly lower for patients with mPGES-1-positive tumors than for those with mPGES-1-negative tumors.

Conclusions

mPGES-1 protein may have an important role in vessel invasion, and may be a prognostic marker in CRC.

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