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**Original contribution**

# Clinical significance of prostaglandin E synthase expression in gastric cancer tissue☆

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**Summary** Studies have linked microsomal prostaglandin E synthase (mPGES)–1 with gastric cancer. The purpose of this study was to determine mPGES-1, mPGES-2, and cytosolic PGES (cPGES) expression in gastric cancer and to evaluate the correlation between mPGES-1 and mPGES-2 expression and clinicopathological factors and cyclooxygenase-2 expression. PGES protein expression was examined by Western blot in gastric cancer cell lines and in biopsy samples from patients with gastric cancer. mPGES-1, mPGES-2, and cPGES protein localizations were examined immunohistochemically in 129 archival gastric cancer surgical resections. mPGES-1 protein expression was found in gastric cancer biopsies and cancer cell lines with differentiated or undifferentiated adenocarcinoma. There was no mPGES-1 expression in nonneoplastic biopsies. All cell lines and tissue samples expressed mPGES-2 and cPGES. Immunohistochemical analysis showed cancer cells expressed mPGES-1 in 47% of cases. mPGES-2 immunoreactivity was seen both in nonneoplastic glandular epithelium and cancer cells; however, cancer cell immunoreactivity was significantly more pronounced in 29% of cases. cPGES expression was constitutive both in nonneoplastic and neoplastic tissues, with no significant variation among cases. mPGES-1 and mPGES-2 expression correlated with cyclooxygenase-2 expression. mPGES-1 and mPGES-2 expression, and tumor-node-metastasis stage had independent prognostic significance under multivariate analysis in patients with gastric cancer overall and in patients with differentiated cancers. However, only tumor-node-metastasis stage and mPGES-2 expression retained independent prognostic significance in patients with poorly differentiated cancers. mPGES-1 and mPGES-2 correlate with clinicopathological factors and may be valuable prognostic factors in gastric cancer.


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## 1. Introduction

Observational and controlled epidemiological studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) modulate cancers of the gastrointestinal tract [1,2]. In vitro studies and animal models of gastrointestinal diseases also support the anticancer properties of NSAIDs [3,4]. Studies have largely attributed the chemopreventive effect of NSAIDs to their suppression of prostaglandin (PG) synthesis through the inhibition of cyclooxygenase (COX, EC 1.14.99.1), the key enzyme in the synthesis of all PGs and the main target of NSAIDs [5]. One of 2 isoforms of the COX enzyme is the inducible COX-2, and its expression has been associated with the upregulation of neoplastic or suppression of antineoplastic factors that modulate tumor progression [6,7]. Human stomach tumors that overexpress COX-2 have been shown to produce more PGE2 than their associated normal mucosa or submucosa [8], and PGE2 has been shown to enhance the survival and invasiveness of cancer cells [9,10] and to inhibit immune surveillance [11], thereby contributing to progression of a variety of cancer cells. These studies clearly point to a central role for COX-2 in human cancers of the digestive tract, in part through its upregulation of PGE2 synthesis. However, there have also been studies suggesting that even selective COX-2 inhibitors can suppress tumor progression via COX-independent pathways that might limit or exclude the role of PGE2 in tumorigenesis [12]. Thus, the role of PGE2 itself in the development of gastric carcinoma is still far from being elucidated.

Recently, 3 different PGE synthases (PGESs, EC 5.3.99.3) have been identified—1 cytosolic and 2 microsomal isomerases—that work downstream of COX for PGE2 synthesis [13-15]. The cytosolic PGES (cPGES) is constitutively expressed in a variety of homeostatic cells and selectively couples with COX-1 in the biosynthesis of PGE2 [14]. In contrast, the microsomal PGES (mPGES)–1 is induced in the course of various pathologies and couples with COX-2 in marked preference to COX-1 [16-19]. The other microsomal enzyme, mPGES-2, although normally constitutively expressed [15], has also been shown to be overexpressed in colorectal adenocarcinoma [20,21] and can couple with either COX isoform, with some preference for COX-2 [18,20]. Several studies have already shown that mPGES-1 is expressed in various cancers that also express COX-2 [22-24], suggesting that the COX-2/mPGES-1/PGE2 pathway is linked to the genesis and growth of cancers. However, there have been few studies targeting PGES expression in either gastric adenoma or cancer [25-28]; and no report has clearly connected mPGES-1 to the clinicopathological features of these diseases. Furthermore, the role of the second microsomal PGES, mPGES-2, in human gastric adenocarcinoma has yet to be explored.

The purpose of the present study is to examine the expression and localization of each of the 3 PGESs in human gastric cancer tissue, to determine their correlation to COX-2 expression and clinicopathological features, and to evaluate their prognostic value in patients with gastric cancer.

## 2. Materials and methods

### 2.1. Materials

Antihuman mouse monoclonal antibody against mPGES-1 and antihuman rabbit polyclonal antibodies (pAbs) against mPGES-1, mPGES-2, and cPGES were all from Cayman Chemical Co (Ann Arbor, MI). Antihuman rabbit pAb against cPGES was compliments of Professor Ichiro Kudo (Showa University, Tokyo, Japan). Recombinant human mPGES-1 protein, prepared as microsomal proteins of *Escherichia coli* transfected with mPGES-1 complementary DNA, was kindly donated by Japan Tobacco Inc, Central Pharmaceutical Research Institute (Osaka, Japan). For Western blot analysis, we used Precision Plus Protein Standard from Bio-Rad Laboratories (Hercules, CA) and Hybond polyvinylidene diflouride membrane and enhanced chemiluminescence reagent from Amersham (High Wycombe, UK). Immunohistochemistry reagents were all from Vector Laboratories, Inc (Burlingame, CA). For cell cultures, we used the RPMI 1640 medium, the Eagle minimal essential medium, and the Dulbecco modified Eagle medium from Nikken Biomedical Laboratory (Kyoto, Japan); penicillin-streptomycin and insulin from Invitrogen Japan KK (Tokyo, Japan); and fetal calf serum from Trace Biosciences Pty Ltd, (New South Wales, Australia).

### 2.2. Cell lines and culture

Cell lines were all derived from human gastric carcinomas. MKN7, MKN74, and MKN28 originated from differentiated carcinomas, MKN45 and FU97 from poorly differentiated carcinomas, and KATO III from signet ring cell carcinoma; all were from the Japanese Collection of Research Bioresources Cell Bank (Tokyo, Japan). Gastric cancer cells ($1X10^5$) were cultured on 10 cm dishes in 10 mL medium with 50 IU/mL penicillin-streptomycin supplemented with 10% fetal calf serum. MKN cells were cultured in RPMI 1640, FU97 cells in the Dulbecco modified Eagle medium (4.5g/L glucose) supplemented with 10 mg/L insulin, and KATO III cells in 45% RPMI 1640 and 45% Eagle minimal essential medium. Cultured cells were incubated at 37°C in a 5% $CO_2$ humidified incubator. Confluent cells were harvested, and lysate protein was used for Western blot analysis.

### 2.3. Biopsy samples

For Western blot analysis, we used 64 gastric biopsy samples (4 biopsies per patient: 2 gastric cancer and 2