15-Hydroxyprostaglandin Dehydrogenase Is Down-regulated in Gastric Cancer

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Abstract

Purpose: We have investigated the expression and regulation of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in gastric cancer.

Experimental Design: Clinical gastric adenocarcinoma samples were analyzed by immunohistochemistry and quantitative real-time PCR for protein and mRNA expression of 15-PGDH and for methylation status of 15-PGDH promoter. The effects of interleukin-1β (IL-1β) and epigenetic mechanisms on 15-PGDH regulation were assessed in gastric cancer cell lines.

Results: In a gastric cancer cell line with a very low 15-PGDH expression (TMK-1), the 15-PGDH promoter was methylated and treatment with a demethylating agent 5-aza-2'-deoxycytidine restored 15-PGDH expression. In a cell line with a relatively high basal level of 15-PGDH (MKN-28), IL-1β repressed expression of 15-PGDH mRNA and protein. This effect of IL-1β was at least in part attributed to inhibition of 15-PGDH promoter activity. SiRNA-mediated knockdown of 15-PGDH resulted in strong increase of prostaglandin E₂ production in MKN-28 cells and increased cell growth of these cells by 31% in anchorage-independent conditions. In clinical gastric adenocarcinoma specimens, 15-PGDH mRNA levels were 5-fold lower in gastric cancer samples compared with paired nonneoplastic tissues (n = 26) and 15-PGDH protein was lost in 65% of gastric adenocarcinomas (n = 210).

Conclusions: 15-PGDH is down-regulated in gastric cancer, which could potentially lead to accelerated tumor progression. Importantly, our data indicate that a proinflammatory cytokine linked to gastric carcinogenesis, IL-1β, suppresses 15-PGDH expression at least partially by inhibiting promoter activity of the 15-PGDH gene.

The incidence of gastric cancer has steadily declined in Western countries, but it still remains one of the leading causes of cancer-related deaths worldwide (1). There are two main histologic types of gastric adenocarcinoma, the intestinal and the diffuse type, which differ in their epidemiologic, etiologic, and genetic properties (2, 3). Curative treatment of gastric cancer requires complete removal of the neoplastic tissue including lymphadenectomy, but even in the case of curative intent, the prognosis is poor and the 5-year survival is only 20% to 30%.

Cyclooxygenase-2 (COX-2) enzyme is critical for the conversion of arachidonic acid to prostanoids in gastrointestinal tumors (4). The major prostanoid produced by epithelial-derived tumor cells is prostaglandin E₂ (PGE₂), which is thought to play an important role in carcinogenesis via modulation of angiogenesis, invasion, and metastasis (5, 6). We and others have shown that expression of COX-2 is elevated in gastric preinvasive dysplasias and in invasive carcinomas (7–9). Importantly, COX-2 is an independent prognostic factor in gastric adenocarcinomas (10). However, due to the increased risk for cardiovascular events (11–13), the use of selective COX-2 inhibitors is not recommended in cancer prevention.

PGE₂ levels are also regulated by degradation and an important enzyme in prostaglandin catabolism is 15-hydroxy-prostaglandin dehydrogenase (15-PGDH). It is a ubiquitously expressed enzyme, which catalyzes the oxidation of 15(S)-hydroxyl group of prostaglandins resulting in 15-keto metabolites with greatly reduced biological activity (14). Recent studies suggest a tumor suppressor activity of 15-PGDH in colon, breast, and lung cancers, showing that 15-PGDH mRNA and protein expression are down-regulated or lost in these
Translational Relevance

Cyclooxygenase-2 (COX-2) is a marker of poor prognosis in gastrointestinal carcinomas, and selective inhibitors of COX-2 have been shown to prevent tumor progression. However, use of these inhibitors has been associated with cardiovascular side effects. Therefore, a more profound understanding of the prostanoid synthesis and degradation pathways is important, which could provide alternative means to interfere with this pathway. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is an enzyme that catalyzes and inactivates prostaglandin E2, and it has recently been described as a tumor suppressor. We used the largest gastric cancer material thus far to show that 15-PGDH is lost in approximately two thirds of gastric adenocarcinomas. Importantly, the proinflammatory cytokine IL-1β seems to play a dual role in gastric cancer, whereas it up-regulates COX-2 expression it down-regulates 15-PGDH expression leading to increased prostaglandin E2 output. Thus, a therapy that reactivates 15-PGDH expression could provide means to treat gastric cancer patients.

Materials and Methods

Experiments in cell lines

Reagents. Interleukin-1β (IL-1β; 10 ng/ml) was purchased from R&D Systems; NS-398 (5 μmol/L) and CAY10397 (10-300 μmol/L) from Cayman; indomethacin, 5-aza-2'-deoxycytidine (5 μmol/L), and trichostatin A (500 ng/mL) from Sigma; and IL-1 receptor antagonist (IL-1ra; 50 ng/mL) was purchased from Santa Cruz. Reagents were incubated with the cells for 24 to 72 h if not mentioned differently and PBS-0.1% precipitation assay buffer or with a Protein/RNA extraction kit (Macherey Nagel). The bisulfite modification was done with the EZ DNA Methylation-Gold kit (Zymo Research). The primer pairs to detect methylated (M) or unmethylated (U) bisulfite converted promoter DNA were as follows: M(forward) 5-CCATTTTGCGCCCTGTGCGGATGCGGGAGGTGCAC-3 (195 bp; ref. 25). Methylation-specific PCR (MSP) was done in a total volume of 50 μL using the EpiTect MSP kit (Qiagen). After denaturation at 95°C for 10 min, 40 to 45 cycles were done as follows: 94°C for 15 s, 56°C for 30 s, and 72°C for 30 s. A final extension was done for 10 min at 72°C. The products were separated on a 1.5% agarose gel and detected by ethidium bromide staining.

15-PGDH protein expression in MKN-1, MKN-7, MKN-45, TMK-1, and MKN-28 cells were analyzed by Western blot analysis. Proteins were extracted in radioimmunoprecipitation assay buffer or with a Protein/RNA extraction kit (Macherey Nagel) and subjected to Western blot analysis as described previously (23). As primary antibodies rabbit antiserum against human 15-PGDH (1:10,000; ref. 24), goat polyclonal anti-human-COX-2 (1:1,000), or anti-β-actin (1:500, both Santa Cruz) were used. Secondary antibodies were goat anti-rabbit (1:2,000; Dako) or donkey anti-goat (1:2,000; Santa Cruz).

A Quantitative real-time PCR. RNA was extracted with the TRIzol Reagent (Invitrogen) or Protein/RNA extraction kit (Macherey Nagel). One microgram of total RNA was converted to cDNA, and quantitative real-time PCR (qRT-PCR) reactions were done and analyzed with the 7500 Fast Real-time PCR system by use of TaqMan gene expression assays for 15-PGDH, COX-2, and Slug (Applied Biosystems) as described earlier (23). Each sample was measured in triplicate and human 18S rRNA served as an endogenous control.

Isolation and bisulfite treatment of DNA. Genomic DNA of gastric cancer cell lines was isolated with the NucleoSpin Tissue kit (Macherey Nagel). The bisulfite modification was done with the EZ DNA Methylation-Gold kit (Zymo Research).

Methylation-specific PCR. The primer pairs to detect methylated (M) or unmethylated (U) bisulfite converted 15-PGDH promoter DNA were as follows: M(forward) 5-CCATTTTGCGCCCTGTGCGGATGCGGGAGGTGCAC-3 (195 bp; ref. 25). Methylation-specific PCR (MSP) was done in a total volume of 50 μL using the EpiTect MSP kit (Qiagen). After denaturation at 95°C for 10 min, 40 to 45 cycles were done as follows: 94°C for 15 s, 56°C for 30 s, and 72°C for 30 s. A final extension was done for 10 min at 72°C. The products were separated on a 1.5% agarose gel and detected by ethidium bromide staining.

-5-Aza-2'-deoxycytidine and trichostatin A treatment. Cells at 15% confluency were treated with 5 μmol/L. 5-Aza-2'-deoxycytidine for 72 h. The medium containing the reagent was renewed every 24 h. Trichostatin A (500 ng/mL) was added for the last 6 h of the experiment.

siRNA treatment. Cells were plated on 12- or 6-well culture dishes and transfected with 20 to 40 nmol/L Drhamacon ON-TARGETplus siRNA targeting 15-PGDH (UUGAGACGCCGCCTGUGUAU, NM_000860), a pool of four duplexes targeting COX-2 (NM_00936), or Slug (NM_003068) or control nontargeting siRNA pool (all Thermo Fisher Scientific) with lipofectamine 2000 (Invitrogen) for 48 to 72 h.

Transfection of reporter constructs and promoter analysis. The 15-PGDH promoter construct (-2,368 to -7 bp) in a pGL3-Basic vector was cotransfected with reporter constructs, and a pool(allThermoFisherScientific)withlipofectamine2000(Invitrogen) or Protein/RNA extraction kit (Macherey Nagel). One microrgram of total RNA was converted to cDNA, and quantitative real-time PCR (qRT-PCR) reactions were done and analyzed with the 7500 Fast Real-time PCR system by use of TaqMan gene expression assays for 15-PGDH, COX-2, and Slug (Applied Biosystems) as described earlier (23). Each sample was measured in triplicate and human 18S rRNA served as an endogenous control.

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Cells were seeded in 96-well plates (200,000 viable cells) were measured using the CellTiter-Blue Assay (Promega). Cells were treated with 40 nmol/L siRNA targeting 15-PGDH, control siRNA, or with Lipofectamine only for 1, 3, 6, and 8 d. Relative numbers of viable cells were stained with Giemsa (Sigma) and colonies were counted.

Pictures were analyzed with ImageJ Software (cutoff, colonies > 600 cells). Cells (1 × 10^4 per dish) were suspended 48 h after which total proteins were lysed in passive lysis buffer and luminescence was measured (Dual-Luciferase Reporter Assay System; Promega). Firefly luciferase values were normalized to control Renilla luciferase reporter pRL-SV40 (Promega; Firefly luciferase reporter was a kind gift of Dr. Gellersen, Endokrinologikum, Hamburg, Germany). Remilia luciferase reporter pRL-SV40 (Promega) served as transfection control. For transient transfection, equimolar amounts of 15-PGDH promoter construct or pGL3-Basic vector (0.8 μg, adjusted with the promoterless plasmid pRNP3) were cotransfected with the Remilia construct (0.004 μg) with Lipofectamine 2000 (Invitrogen). The transfection medium was removed after 4 h, and the cells were grown for 18 h in serum-reduced medium (0.5% serum). IL-1β (10 ng/mL) or control were added for 6 h after which total proteins were exposed in BAS-2500 phosphoimager and the signal was normalized to glyceraldehyde-3-phosphate dehydrogenase using L Process and MacBas software (Fujifilm).

Experiments in tissue samples

Dot blot analysis. Cancer profiling array I containing normalized cDNA samples of gastric tumors and matched nonneoplastic tissues (n = 26) was purchased from BD Biosciences Clontech. 15-PGDH probe containing bases 314-1027 of human 15-PGDH (NM_000860) was produced from human cDNA (reverse transcribed RNA from SW480 cells) using random primers) using the forward primer 5'-GCGTTGGCTAATCTTATGA-3' and reverse primer 5'-GTCGCCACATCA-3. The PCR product was TA cloned into pCRII vector (Invitrogen) and the EcoRI fragment was gel purified,32P labeled, and hybridized to the filter. To quantify 15-PGDH expression, the filter was exposed in BAS-2500 phosphoimager and the signal was normalized to that of glyceraldehyde-3-phosphate dehydrogenase using L Process and MacBas software (Fujifilm).

Tissue microarrays and immunohistochemistry. Specimens for the tissue microarray were derived from a set of 327 consecutive histologically verified gastric adenocarcinoma patients operated at the Department of Surgery, Helsinki University Central Hospital between 1983 and 1999. Clinicopathologic data were collected from patient records. Tumors were classified according to the Union Internationale Contra Cancrum classification of 1992. Survival data were obtained from patient records, the Finnish Cancer Register, and Statistics Finland. The approval of the study was obtained from the local ethics committee. This material has been described in a previous study from where more detailed clinical information can be obtained (10). We were able to successfully obtain tissue microarray cores, immunostain, and score 210 samples for 15-PGDH and 240 for COX-2.

Fig. 2. CpG methylation status in gastric cancer cells and effect of demethylation and histone acetylation on 15-PGDH expression. A, MSP analysis of gastric cancer cell lines with primer pairs detecting methylated (M) or unmethylated (U) 15-PGDH promoter DNA. Completely unmethylated (Ctr) or methylated (C) bisulfite converted human DNA served as control. B, treatment with the demethylating 5-Aza-2'-deoxycytidine (Aza; 5 μmol/L) for 72 h. Addition of the histone deacetylase inhibitor trichostatin A (TSA; 6 h; 500 ng/mL) for 6 h. Columns, means from two independent experiments (n = 6); bars, SE; *, P ≤ 0.01 versus control; **, P ≤ 0.01 versus trichostatin A and versus 5-Aza-2'-deoxycytidine. C, expression of Slug mRNA in MKN-28, MKN-7, and TMK-1 gastric cancer cells as measured by qRT-PCR. Expression levels are shown relatively to MKN-28 (relative expression, 1; n = 3). D, Slug siRNA inhibited Slug mRNA expression in MKN-7 cells after 72 h incubation (left), but did not alter 15-PGDH mRNA expression (right). Columns, means from two independent experiments (n = 5); bars, SE; *, P = 0.008 versus control.
microarrays were constructed from formalin-fixed and paraffin-embedded archive specimens as described previously (27). In brief, representative tumor areas were selected from H&E-stained sections, and three tissue cores (each diameter 0.6 mm) from different parts of the specimen from each tumor block were placed into a recipient paraffin block. The presence of cancer cells was verified on H&E-stained sections. The immunostaining protocol for human specimens was carried out as described previously (28). Specimens were immunostained with a rabbit antiserum against human 15-PGDH (1:5,000; ref. 24) or a COX-2–specific anti-human mouse monoclonal antibody (1:200 Cayman).

**Evaluation of 15-PGDH and COX-2 immunostaining.** 15-PGDH and COX-2 immunohistochemical stainings were independently scored by two investigators (AT and AR for 15-PGDH, JM and AR for COX-2), and in the case of discrepant scores, the consensus score served for further analysis. The following scoring criteria for the tumor cells were agreed upon before the analysis: 0, no staining; 1, weak diffuse cytoplasmic staining (may show stronger intensity in <10% of the cancer cells); 2, moderate to strong cytoplasmic staining in 10% to 90% of the cancer cells; 3, over 90% of the tumor cells stained with strong intensity. In statistical analysis, 15-PGDH and COX-2 scores were divided into two groups (15-PGDH negative 0 versus positive 1-3; COX-2 absent/low 0-1 versus high 2-3).

**RNA and DNA extraction from fresh frozen tissue samples.** Fresh frozen gastric tissue samples were stored at -70°C, and the histology was determined from frozen sections. Samples were then stored in RNA Later-ICE reagent (Ambion) at -80°C. For nucleic acid extraction, the samples were homogenized in RLT buffer of the RNeasy midi kit (Qiagen) using Ultra-Turrax homogenizer (IKA Works). After homogenization, the RNA was extracted with the RNeasy midi kit and the DNA with the DNeasy tissue extraction kit (Qiagen). DNA and RNA were available from nonneoplastic gastric tissues (n = 3, nonneoplastic samples 6 and 32 derived from patients with gastric carcinoma, sample 33 from a patient with pancreatic carcinoma) and from intestinal type gastric adenocarcinoma samples (n = 18). Intestinal gastric carcinoma tissue samples were chosen to obtain tumor samples with a high proportion of tumors cells versus stromal cells. To determine mRNA expression levels of 15-PGDH, qRT-PCR was done as describe above. The DNA was first treated with bisulfite followed by MSP analysis as described above.

**Statistical analysis.** The data were analyzed by χ² test (clinicopathologic parameters), Kaplan-Meier method and log-rank test (life tables of overall survival), paired t test (dot blot analysis), Spearman’s rank correlation (dot by dot analysis), and nonparametric Mann-Whitney U test or unpaired t test for the rest of the analysis. A P value of ≤0.05 was considered statistically significant (SPSS 16.0) and all data are shown as means ± SE.

**Results**

**15-PGDH expression in gastric cancer cells.** We first investigated expression of 15-PGDH in gastric cancer cell lines that originated from diffuse (MKN-45, TMK-1) and intestinal type (MKN-7, MKN-28) adenocarcinomas and from an adenosquamous carcinoma (MKN-1; ref. 22). No clear association was found in respect of the origin of the tumor because 15-PGDH mRNA and protein were found to be expressed in both intestinal (MKN-28) and in diffuse type (MKN-45) cancer cells as well as in the adenosquamous type (Fig. 1). Importantly, two of the cell lines (TMK-1 and MKN-7) expressed very low levels of 15-PGDH.
15-PGDH expression is modulated epigenetically in gastric cancer cells. To understand the mechanism of almost undetectable levels of 15-PGDH expression in TMK-1 cells, we determined the methylation status of the 15-PGDH promoter in every gastric cancer cell line. Our results indicate that the 15-PGDH promoter is methylated in TMK-1 cells, but not in any of the other gastric cancer cell lines (Fig. 2A). TMK-1 cells were treated with a reagent (5-aza-2'-deoxycytidine) that inhibits DNA methylation. This treatment increased expression of 15-PGDH mRNA by 3.2-fold (Fig. 2B). We also investigated another epigenetic gene regulatory mechanism by using a histone deacetylase inhibitor trichostatin A, which led to a 1.5-fold increase of 15-PGDH mRNA expression after 6 hours of treatment (Fig. 2B) and to a 3.0-fold increase after an incubation period for 24 hours (data not shown). When trichostatin A was added together with 5-aza-2'-deoxycytidine, a synergistic 6.7-fold increase in 15-PGDH expression was evident (Fig. 2B). EMT-related transcription factors have been...
shown to regulate 15-PGDH promoter and particularly Slug-suppressed 15-PGDH expression in non–small cell lung cancer cells (29–31). In the other gastric cancer cell line with low basal 15-PGDH expression, i.e., MKN-7 cells, we observed almost 300-fold higher expression level of Slug transcript when compared with MKN-28 or TMK-1 cells (Fig. 2C). However, although Slug-targeted siRNA successfully suppressed expression of Slug (Fig. 2D, left), it did not modulate 15-PGDH mRNA expression (Fig. 2D, right).

**IL-1β inhibits 15-PGDH expression in gastric cancer cells.** Next, we investigated regulation of 15-PGDH expression in the MKN-28 cell line that had the highest basal expression of this enzyme. The proinflammatory cytokine IL-1β has been reported previously to play an important role in gastric carcinogenesis (32, 33). IL-1β inhibited 15-PGDH protein expression in MKN-28 cells as determined by Western blot analysis (Fig. 3A, left). Similarly, IL-1β inhibited 15-PGDH expression in MKN-1 cells (Fig. 3A, left). IL-1ra blocked the IL-1β–mediated down-regulation of 15-PGDH expression, but had no effect on basal 15-PGDH expression (Fig. 3A, right). In contrast to IL-1β, COX-2 inhibitor NS-398 or an unspecific COX inhibitor indomethacin did not have an effect on 15-PGDH protein levels (Fig. 3B, left). In addition, treatment with COX-2 siRNA did not alter the expression of 15-PGDH protein, whereas expression of COX-2 was decreased (Fig. 3B, right). To address the mechanism of IL-1β–mediated inhibition of 15-PGDH expression, we first investigated the effect of IL-1β on 15-PGDH mRNA levels as detected by qRT-PCR. Our results show that IL-1β decreased steady-state levels of the 15-PGDH transcript in MKN-28 cells (Fig. 3C, left). We then measured transcriptional activity of a 15-PGDH promoter-reporter construct, and our data indicate that IL-1β suppressed the promoter activity by 34% (Fig. 3C, right).

**15-PGDH inhibition leads to increased PGE2 production and promotes anchorage-independent growth.** To test whether 15-PGDH is biologically active in gastric cancer cells, we used an siRNA approach to inhibit its expression. Treatment with 15-PGDH siRNA resulted in a clear decrease of 15-PGDH protein expression (Fig. 4A) and a 70% inhibition of 15-PGDH mRNA expression in MKN-28 cells (Fig. 4B, left). Importantly, 15-PGDH enzyme is biologically active in these cells as inhibition of its expression led to a 5.7-fold increase in production of PGE2 as shown by treatment with 15-PGDH siRNA (Fig. 4C, left). Similarly, treatment with a small molecular inhibitor (Cay10397) of 15-PGDH stimulated PGE2 production (Fig. 4C, right). Increased production of PGE2 is unlikely to be dependent on COX-2 enzyme because expression of COX-2 mRNA did not change after the treatment with 15-PGDH siRNA (Fig. 4B, right). When the cells were grown in an anchorage-independent fashion on soft agar, the cells treated with 15-PGDH siRNA formed 31% more colonies than the control cells (Fig. 4D, left). However, no difference in proliferation rate was observed when the cells were grown in culture plates (Fig. 4D, right).

**Expression of 15-PGDH in human gastric cancer tissues.** Finally, we wanted to explore the role of 15-PGDH in clinical gastric cancer specimens. Analysis of 15-PGDH mRNA expression revealed a 5.0- ± 0.59-fold higher transcript level in nonneoplastic tissues when compared with matched gastric adenocarcinoma specimens as detected by a dot blot analysis (Fig. 5A and B). Next, we immunostained a set of 210 gastric adenocarcinoma samples with an antibody against 15-PGDH, and found that 65% of the tumors had completely lost 15-PGDH expression, and representative staining results are shown in Fig. 5C. The specimens negative for 15-PGDH expression were intestinal (48%) and diffuse (52%) type gastric adenocarcinomas. Of the tumors expressing 15-PGDH, 54% were intestinal and 46% diffuse type adenocarcinomas. In contrast to COX-2 (10), no association between 15-PGDH expression and clinicopathologic parameters or overall survival was found (data not shown). However, when we did a dot by dot comparison of 15-PGDH and COX-2 expression, we found that the subgroup of specimens completely negative for COX-2 was the only category of COX-2 staining pattern that contained samples devoid of any expression for 15-PGDH. In contrast, dots with high (score 3) expression of 15-PGDH showed exclusively a moderate to high expression of COX-2 (Fig. 5D). However, no correlation between 15-PGDH and COX-2 expression in this dot by dot analysis was found ($P = 0.920$).
To investigate the significance of promoter methylation in clinical cancer samples, we analyzed the 15-PGDH promoter methylation status in 3 nonneoplastic gastric samples and in 18 intestinal type gastric cancer specimens. Methylated promoter could only be detected in one cancer sample and very weak products were observed in two additional cancer samples after 40 PCR cycles (Fig. 6A). When we reanalyzed the DNA samples with 45 PCR cycles, we were able to detect methylated promoter in one nonneoplastic sample and in nine gastric cancer specimens (Fig. 6B). We next analyzed expression of 15-PGDH mRNA, but found no statistically significant difference in expression of 15-PGDH when samples showing promoter methylation were compared with the ones without any methylation ($P = 0.53$; Fig. 6C). Interestingly, the sample with the highest methylation signal (sample 31; Fig. 6A) was the one with the lowest 15-PGDH mRNA expression level in this set.

**Discussion**

IL-1$\beta$ is an important proinflammatory cytokine that has been linked to gastric carcinogenesis (32, 33). Our results show for the first time that IL-1$\beta$ suppresses expression of 15-PGDH in gastric cancer cells. Mechanism behind this inhibition is due to modulation of transcription of the 15-PGDH gene, which is based on our experiments with the promoter-reporter construct (Fig. 3C). Importantly, suppression of 15-PGDH expression either by siRNA oligos or by a small molecular inhibitor lead to an increase in PGE$_2$ production, which shows that this enzyme is functionally active in gastric cancer cells and modulation of its expression or activity translates to altered prostaglandin output (Fig. 4C). Finally, gastric cancer cells that were transiently silenced for 15-PGDH formed a higher number of colonies in anchorage-independent conditions (Fig. 4D).

Expression of 15-PGDH has been shown to be lost or decreased in 36% to 94% of colon, lung, breast, and bladder carcinomas (15–18, 20, 34). Previously, it was shown that 77% to 80% of the gastric cancer specimens lacked 15-PGDH protein expression as detected by immunohistochemistry (15, 35), although in one report, 15-PGDH protein expression was lost only in 10% of the gastric cancer samples (36). Our results are consistent with the data that show clear reduction in 15-PGDH expression in gastric cancer because 15-PGDH mRNA expression was reduced by 5-fold in tumor samples when compared with paired nonneoplastic ones. In addition,
our data show that 65% of the gastric cancer specimens were negative for 15-PGDH protein. However, unlike Liu et al. (35), where 15-PGDH expression correlated with tumor-node-metastasis stage, lymph node metastasis, and histologic type of the tumor, we did not find any association with 15-PGDH immunostaining and clinicopathologic parameter or prognosis. This is in contrast to COX-2 that was found to be an independent prognostic factor in this series of gastric cancer specimens (10). On the other hand, our clinical data suggest that whenever a gastric tumor expresses 15-PGDH, it also needs to express relatively high COX-2 levels (Fig. 5D). Because COX-2-derived PGE\textsubscript{2} has been linked to angiogenesis, invasion, and metastasis (6), suppression of 15-PGDH can promote carcinogenesis (17–19, 21). Thus, tumors that retain high 15-PGDH expression and are able to catabolize PGE\textsubscript{2} may need to counteract this with increased COX-2 expression to maintain adequate PGE\textsubscript{2} levels during tumor progression.

Expression of 15-PGDH is inhibited by IL-1\textbeta in MKN-1 and MKN-28 gastric cancer cells (Fig. 3A). IL-1ra blocked the effect of IL-1\textbeta, but did not affect basal 15-PGDH expression. This is most likely due to the fact that the endogenous expression of IL-1\textbeta is very low in gastric cancer cell lines (37). In a clinical situation, this could mean that cancer cells are targets of IL-1\textbeta produced by neighboring stromal cells and not by cancer cells themselves. Our finding is in line with a general theme that indicates that carcinogenesis-promoting factors suppress 15-PGDH expression, including epidermal growth factor in colorectal and lung cancer cells (16, 29, 30). As previously shown for epidermal growth factor in colon cancer (29), IL-1\textbeta inhibited transcription of the 15-PGDH promoter in MKN-28 cells. Previously, IL-1\textbeta has been shown to reduce 15-PGDH expression in lung cancer cells via induction of COX-2 expression (38), and a link between COX-2 and 15-PGDH regulation was reported in SGC7901 gastric cancer cells (35). In contrast, we were unable to find any relation between COX-2 and 15-PGDH in MKN-28 gastric cancer cells, which is supported by our data that show that treatment with COX inhibitors or COX-2 siRNA did not alter 15-PGDH expression. In addition, treatment with 15-PGDH siRNA had no effect on COX-2 expression. Finally, we did not find any correlation between COX-2 and 15-PGDH expression in our clinical material.

15-PGDH expression has been shown to be modulated by epigenetic means such as methylation and histone modification (18, 39). Our data show that DNA methylation and histone deacetylation suppressed 15-PGDH mRNA expression in TMK-1 cells, which might partly explain very low basal levels of 15-PGDH in this cell line. In gastric cancer samples, however, strong 15-PGDH promoter methylation seems to be a rare event. Only one sample showed a methylation signal equally strong to the unmethylated promoter, which indeed had the lowest level of 15-PGDH mRNA expression. However, when the samples that showed any 15-PGDH promoter methylation were compared with samples without any methylation, there was no significant difference in 15-PGDH mRNA expression levels. A similar finding was reported in prostate cancer samples, where 15-PGDH promoter methylation was not accompanied by decreased gene expression and even the 15-PGDH promoter in normal epithelial cells was strongly methylated (25). In breast cancer tissues, however, samples that had a methylated 15-PGDH promoter region expressed reduced levels of 15-PGDH mRNA compared with unmethylated tumors (18).

In this study, we examined the expression of 15-PGDH in gastric cancer specimens as well as in gastric cancer cell lines. We show that 15-PGDH is significantly down-regulated in gastric cancer specimens. Epigenetic mechanisms are at least in part responsible for the low basal expression of 15-PGDH in TMK-1 gastric cancer cells but promoter methylation does not seem to be the main suppressing factor of 15-PGDH expression in human gastric tissue samples. Importantly, we found that IL-1\textbeta is a transcriptional repressor of 15-PGDH promoter in MKN-28 cells. Thus, IL-1\textbeta plays a dual role in gastric cancer by inhibiting 15-PGDH and by up-regulating COX-2 (40), which both lead to increased output of tumor promoting prostanooids.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

14. Tai HH, Ensor CM, Tong M, Zhou H, Yan F. Prosta
glandin dehydrogenase, a COX-2 oncogene antagonist, is a TGF-\beta-induced suppressor of human gastrointestinal cancers. Proc Natl Acad Sci USA 2004;101:7468–73.
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