Expression of 15-PGDH is downregulated by COX-2 in gastric cancer

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Introduction

Gastric cancer is one of the most common malignant tumors in China. Overexpression of cyclooxygenase-2 (COX-2) was frequently detected in gastric cancer and was believed to play a crucial role in gastric carcinogenesis (1–10). COX-2 could inhibit apoptosis of tumor cell and promote proliferation, migration of gastric cancer cells and angiogenesis of gastric cancer. Upregulation of COX-2 might facilitate invasion of gastric cancer and was significantly related to the low survival rate of gastric cancer patients (11–14). COX-2, which can be induced by lipopolysaccharide, inflammatory cytokines, growth factors and some tumor promoters, is the key enzyme to catalyze the synthesis of prostaglandins (15,16). Overexpression of COX-2 led to the accumulation of prostaglandins, particularly prostaglandin E2, play an important role in gastric cancer and its level is controlled not only by synthesis but also by degradation (28). Thus, it is also necessary and crucial to determine the relationship between 15-PGDH, the key enzyme in prostaglandin degradation as the natural COX-2 antagonist, and COX-2, the key enzyme to catalyze the synthesis of prostaglandins, in human gastric cancer.

In the present study, using two-dimensional electrophoresis (2-DE), we found that 15-PGDH was one of the significantly upregulated proteins in gastric cancer SGC7901 cells in which the expression of COX-2 was knocked down by RNA interference. Furthermore, the expression of 15-PGDH was decreased when the expression of COX-2 was upregulated in gastric cancer SGC7901 cells. We demonstrated that 15-PGDH is lowly expressed in human gastric cancer and is significantly negatively correlated with COX-2 by immunohistochemical assay. The same results were also found in tissue samples, using western blotting. The low expression of 15-PGDH was found to be related to the differentiation, tumor, lymph node metastasis (TNM) staging and lymph node metastasis of gastric cancer, using statistical analysis on the results of immunohistochemical studies. All these results of the study indicate that 15-PGDH may be downregulated by COX-2 and act as a tumor suppressor in human gastric cancer. The results presented here will provide clues to the further study of the mechanisms of gastric cancer carcinogenesis and provide a candidate gene for gastric cancer therapy.

Materials and methods

Cell culture

Human gastric adenocarcinoma cell line SGC7901 was obtained from Academy of Military Medical Science (Beijing, China) and preserved in our institute (32). Both cell lines were maintained in RPMI-1640 medium (Gibco Carlsbad, California) and supplemented with 10% heat-inactivated fetal bovine serum (Gibco) and antibiotics in a humidified incubator with a mixture of 5% CO2 and 95% air at 37°C.

Tissue collection

For immunostaining of 15-PGDH and COX-2, paraffin blocks of gastric cancer tissues and adjacent non-tumor tissue specimens from 55 primary gastric cancer patients who received surgical resection in Xijing Hospital were obtained from the Department of Pathology in Xijing Hospital of the Fourth Military Medical University. Paraffin blocks of gastric inflammatory tissues (10 gastritis tissues and 10 gastric ulcer tissues) were also obtained from the Department of
Fig. 1. Two-dimensional gel analyses and MALDI-TOF MS identification of differential expression proteins from SGC7901 cells transfected with COX-2 siRNA or empty pSilencer vector. (A) Analysis of protein expression level for COX-2 in SGC7901-COX-2/siRNA and SGC7901-pSilencer cells by western blotting.

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Pathology in Xijing Hospital. For western blotting, fresh surgical gastric cancer tissues and adjacent non-tumor tissues were obtained from eight patients who underwent surgery in Xijing Hospital. All gastric cancer cases were clinically and pathologically proved. The samples were snap frozen in liquid nitrogen and stored at −80°C until analysis. The study was approved by The Human Research Committee of University and performed with the consent of the patients. Each patient’s age, sex, tumor size, histological type of the neoplasm and TNM staging were obtained from surgical and pathological records.

Small interfering RNA plasmid construction and transfection

Three pairs of hairpin small interfering RNA (siRNA) oligos for COX-2 were designed according to the siRNA design Web site (http://www.ambion.com/techlib/misc/siRNA_finder.html). Target sequences were compared with the human genome database in a BLAST search to ensure that the chosen sequences were not highly homologous to other coding sequences. For oligo-1: sense, 5'-gatccgcaggaagttcatccctgatccttcaagagaggatcagaagggatgtgagagttcatacgattctttttttttttttt-3' and anti-sense, 5'-gactttcaaaaaaaccagggaggtgaggctggctgaacatacatatcgatgggttttttgaa-3'. For oligo-2: sense, 5'-gatccgaagacttcggctagcacagagcagacagagcagaggtgtttcttttttttttttttttt-3' and anti-sense, 5'-gatcggccgagagctgtcttcctctctctctctctctctctctctcagggacgagggagaggtgtgagagttcatacgattcacttctttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
California) according to the manufacturer’s instructions. 3′, 3′-Diaminobenzenedi- ne (Sigma–Aldrich) was used as the chromogen for the immunoperoxidase reaction. In control sections, non-immune goat IgG or mouse IgG was used to substitute for the primary antibodies.

For cell staining, SGC7901-COX-2/siRNA and SGC7901-pSilencer cells and SGC7901-COX-2 and SGC7910-pDNA3.1 cells were cultured on glass coverslips for 24 h and fixed with 4% paraformaldehyde. The fixed cells were rinsed in PBS and permeabilized with 0.1% Triton X-100 in PBS for 5 min. The fixed cells were then stained and examined as tissue sections.

All stained sections and cells were evaluated by two independent investigators in a blind manner. The scoring was based on intensity and extensity. The percentage of positive tumor cells was determined semiquantitatively by assessing the whole tumor section, and each sample was scored on a scale of 0–4, in which 0 was used for positive staining in ≤1% of the cells, 1 in 1–25%, 2 in 26–50%, 3 in 51–75% and 4 in 76–100%. The intensity of immunostaining was determined as 0 (negative staining), 1 (weakly staining), 2 (moderately staining) and 3 (strongly staining). The immunoreactive score was calculated by sum of these two parameters and the final score of ≥3 was considered to be positive.

**Western blotting analysis**

To determine the expression of various proteins in various cells and tissues, western blotting analysis was performed. The cells collected from plates were to extract the proteins in lysis buffer [150 mM NaCl, 50 mM Tris–HCl (pH 8.8), 0.1% sodium dodecyl sulfate, 2 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, 1% NP-40, 5 µg/ml aprotinin and 1 µg/ml leupeptin] at 4°C on ice. Tissues were homogenated in the same lysis buffer to extract the proteins at 4°C on ice. The protein samples were separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and were transferred to nitrocellulose membranes. The membranes were blocked with 10% fat-free milk at room temperature for 2 h and incubated with anti-COX-2 antibody (1:500 dilution), anti-15-PGDH antibody (1:200 dilution) and anti-β-actin antibody (Sigma–Aldrich; 1:2000 dilution) at 4°C overnight. After three washes for 15 min in tris-buffered saline supplemented with 0.1% Tween-20, the membrane was incubated with the horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG as a secondary antibody (Santa Cruz Biotechnology, California; 1:2000 dilution) for 2 h at room temperature, respectively. Enhanced chemiluminescence (ECL kit, Santa Cruz Biotechnology, California; 1:2000 dilution) was used for visualizing the antigens. Western blotting for β-actin was used as an internal control. The bidimensional optical densities of proteins on the films were quantified and analyzed with Molecualr Analyst software (Bio-Rad). Each experiment was repeated three times and the ratios of COX-2 and 15-PGDH against β-actin were calculated.

**Statistical analysis**

For statistical analysis, the statistical tests were performed using SPSS version 11.0 software package (SPSS, Chicago, Illinois). The chi-square test of significance and Fisher’s exact probability calculation were used to compare the differences of groups for immunohistochemical scores of 15-PGDH and COX-2 accumulation with clinical pathological parameters. Correlation between 15-PGDH and COX-2 was analyzed using Spearman rank correlation. Student’s t-test and one-way analysis of variance analysis were adopted for other data. P < 0.05 was considered statistically significant.

**Results**

**Differential expression proteins between SGC7901-COX-2/siRNA and SGC7901-pSilencer**

To analyze differential expression proteins, we transfected SGC7901 cells with COX-2-siRNA plasmid. After G418 selection, western blotting analysis confirmed that the stable clones transfected with COX-2-siRNA showed lower COX-2 expression compared with empty vector-transfected SGC7901-pSilencer cells. Among them, the COX-2 expression of the first stable clone (siRNA1) was the lowest in all clones, so the first stable clone was used to do the further study (Figure 1A).

2-DE was used to identify the differentially expressed proteins in the two cell lines. In the pH range 4–7, 2-DE maps of SGC7901-COX-2/siRNA and SGC7901-pSilencer displayed ~1000 spots each. Most of 2-DE-separated spots shared identical location, shape and density. Two representative 2-DE maps are shown in Figure 1B. Fourteen differentially expressed protein spots that have been identified between the two cell lines are marked with arrows in Figure 1B. The region of the gels showing differentially expressed proteins between SGC7901-COX-2/siRNA and SGC7901-pSilencer is shown in Figure 1C. The relative expression level of differential expression proteins downregulated or upregulated in SGC7901-COX-2/siRNA cells compared with SGC7901-pSilencer are shown in Figure 1D and E. The MALDI-TOF MS map and database query result of spot 9 are shown in Figure 1F. The information of peptide mass fingerprints was searched in the Swiss-Prot database, and the query result showed that protein spot 9 was NAD+–linked-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH). The annotation of 14 differential

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**Table I. MALDI-TOF MS identified proteins in SGC7901-COX-2/siRNA and SGC7901-pSilencer**

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>Accession number</th>
<th>Protein name</th>
<th>Molecular weight (Da)</th>
<th>pI</th>
<th>Expression in SGC7901-COX-2/siRNA</th>
<th>Biological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Q13042</td>
<td>Cell division cycle protein 16 homolog</td>
<td>71 655</td>
<td>5.55</td>
<td>Downregulated&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Regulation of cell cycle</td>
</tr>
<tr>
<td>2</td>
<td>P63261</td>
<td>Actin, cytoplasmic 2</td>
<td>41 661</td>
<td>5.31</td>
<td>Downregulated</td>
<td>Regulation of cell motility</td>
</tr>
<tr>
<td>3</td>
<td>P31947</td>
<td>14-3-3 protein sigma</td>
<td>27 774</td>
<td>4.68</td>
<td>Downregulated</td>
<td>Adapter protein; p53-regulated inhibitor</td>
</tr>
<tr>
<td>4</td>
<td>P08670</td>
<td>Vimentin</td>
<td>53 520</td>
<td>5.06</td>
<td>Downregulated</td>
<td>Structure protein</td>
</tr>
<tr>
<td>5</td>
<td>P11142</td>
<td>Heat shock</td>
<td>70 898</td>
<td>5.37</td>
<td>Downregulated</td>
<td>Chaperone</td>
</tr>
<tr>
<td>6</td>
<td>P50135</td>
<td>Histamine N-methyltransferase</td>
<td>33 295</td>
<td>5.18</td>
<td>Downregulated</td>
<td>Inactivation of histamine</td>
</tr>
<tr>
<td>7</td>
<td>P20337</td>
<td>Ras-related protein Rab-3B</td>
<td>24 757</td>
<td>4.85</td>
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<td>Protein transport</td>
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<tr>
<td>8</td>
<td>Q04760</td>
<td>Lactoylglutathione lyase</td>
<td>20 588</td>
<td>5.25</td>
<td>Upregulated&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>9</td>
<td>P15428</td>
<td>15-Hydroxyprostaglandin dehydrogenase [NAD&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>28 977</td>
<td>5.56</td>
<td>Upregulated</td>
<td>Inactivation of prostaglandins</td>
</tr>
<tr>
<td>10</td>
<td>Q07021</td>
<td>Complement component 1 Q subcomponent-binding protein precursor</td>
<td>31 362</td>
<td>4.74</td>
<td>Upregulated</td>
<td>Unknown</td>
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<tr>
<td>11</td>
<td>Q9UK05</td>
<td>Growth differentiation factor 2 precursor</td>
<td>47 320</td>
<td>6.03</td>
<td>Upregulated</td>
<td>Formation of bone</td>
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<tr>
<td>12</td>
<td>P11021</td>
<td>Heat shock kDa protein 5</td>
<td>72 333</td>
<td>5.01</td>
<td>Upregulated</td>
<td>Chaperone</td>
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<td>13</td>
<td>P48739</td>
<td>Phosphatidylinositol transfer protein beta subform</td>
<td>31 408</td>
<td>6.44</td>
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<td>Protein transport</td>
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<tr>
<td>14</td>
<td>P38646</td>
<td>Stress-70 protein</td>
<td>68 759</td>
<td>5.44</td>
<td>Upregulated</td>
<td>Regulation of cell proliferation</td>
</tr>
</tbody>
</table>

<sup>a</sup>Swiss-Prot accession number.

<sup>b</sup>Downregulated showed that spot intensity ≥2-fold decreased in SGC7901-COX-2/siRNA in comparison with SGC7901-pSilencer; upregulated showed that spot intensity ≥2-fold increased in SGC7901-COX-2/siRNA in comparison with SGC7901-pSilencer.
Expression of 15-PGDH in gastric cancer

The lower expression of COX-2 than in SGC7901-pSilencer (Figure 2A and B). We then transfected SGC7901 cells with the sense expression plasmid for COX-2. After G418 selection, western blotting analysis confirmed that the stable cell clones transfected with pcDNA3.1-COX-2 showed high COX-2 expression compared with empty vector-transfected SGC7901 cells (Figure 2C). Western blotting and immunocytochemical assay showed that the 15-PGDH expression was much lower (51.72%) in SGC7901-COX-2 cells than in SGC7901-pcDNA3.1 cells (Figure 2C and D). These results demonstrated that alteration of COX-2 could lead to an inverse change of 15-PGDH in gastric cancer cell lines and confirmed that COX-2 could regulate the expression of 15-PGDH in vitro, which was consistent with above findings by 2-DE.

Immunohistochemical expression of 15-PGDH and COX-2 in gastric cancer tissues and their relationship with clinicopathological parameters

The 15-PGDH protein staining exhibited weak or even absent expression in gastric cancer tissues with a total immunoreactive negative rate 80.0% (44/55) in gastric cancer tissues whereas the expression of it was significantly higher in adjacent non-tumor tissues than in gastric cancer tissues (P < 0.05) (Figure 3A). Positive immunoreactivity of COX-2 was observed in 76.4% (42/55) of gastric cancer tissues.
Gastric inflammatory tissues were also involved for 15-PGDH expression and we found descending tendency from normal mucosa, inflammatory tissues, to gastric cancer tissues whereas in case of COX-2, we found ascending tendency (Figure 3A). Since the expression of COX-2 and 15-PGDH was contradictory, we analyzed their correlation in gastric cancer. Spearman analysis showed that there was a significant negative correlation between COX-2 and 15-PGDH immunoreactivity with $r_s = -0.564$ ($P < 0.01$) (Table II).

About their relationship with clinicopathological parameters, statistical analysis showed that neither gender nor age was correlated to the expression of 15-PGDH, whereas the level of 15-PGDH was significantly lower in patients of III–IV stage than in I–II stage ($P < 0.05$), its expression had significant difference among differentiation grade ($P < 0.01$). For COX-2, although none of gender, age and differentiation grade was related to its expression, the level of COX-2 showed significant difference among different TNM stage ($P < 0.01$, well versus moderately; $P < 0.05$, well versus poorly) and lymph node metastasis grade ($P < 0.05$) (Table III). The results from immunohistochemistry analysis suggested that 15-PGDH may not only be regulated by COX-2 in gastric cancer but also play a suppressive role independently in tumorigenesis of gastric cancer.

| Table II. Relationship between COX-2 and 15-PGDH expression status in gastric cancer |
|-----------------------------------------------|---------------|----------|
| COX-2 expression                              | 15-PGDH expression |
| Total cases ($n$)                             | Negative | Positive | Total cases ($n$) |
| Negative                                      | 8        | 36       | 44 |
| Positive                                      | 5        | 6        | 11 |
| Total cases ($n$)                             | 13       | 42       | 55 |

$r_s = -0.564$, $P < 0.01$.

The expression of COX-2 and 15-PGDH in gastric cancer tissues and adjacent non-tumor tissues by western blotting assay

Expression levels of COX-2 and 15-PGDH were also examined by western blotting in gastric cancer tissues and adjacent non-tumor tissues taken from eight patients. Each pair of sample was obtained from the same patient. As shown in Figure 3B, 15-PGDH expression was found decreased in gastric cancer tissues, whereas COX-2 was overexpressed. Expression of 15-PGDH in 87.5% tumor tissues showed at least a 2-fold decrease whereas COX-2 expression showed no change.

\[ \text{Expression of COX-2 and 15-PGDH in gastric cancer tissues and adjacent non-tumor tissues by western blotting assay} \]

\[ \text{Expression levels of COX-2 and 15-PGDH were also examined by western blotting in gastric cancer tissues and adjacent non-tumor tissues taken from eight patients. Each pair of sample was obtained from the same patient. As shown in Figure 3B, 15-PGDH expression was found decreased in gastric cancer tissues, whereas COX-2 was overexpressed. Expression of 15-PGDH in 87.5% tumor tissues showed at least a 2-fold decrease whereas COX-2 expression showed no change.} \]
Expression of 15-PGDH in gastric cancer

Table III. Relationship of the immunohistochemical expression of COX-2 and 15-PGDH with clinicopathological parameters in gastric cancer

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Cases (n)</th>
<th>COX-2 (positive)</th>
<th>P value</th>
<th>PGDH (negative)</th>
<th>P value</th>
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<tr>
<td>Total cases (n)</td>
<td>55</td>
<td>42</td>
<td>NS</td>
<td>44</td>
<td>NS</td>
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<tr>
<td>Gender</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>39</td>
<td>29</td>
<td>NS</td>
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<td>NS</td>
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<tr>
<td>Female</td>
<td>16</td>
<td>13</td>
<td></td>
<td>14</td>
<td></td>
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<td>Age (years)</td>
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<td></td>
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<td></td>
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<tr>
<td>&lt;50</td>
<td>18</td>
<td>15</td>
<td>NS</td>
<td>13</td>
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<tr>
<td>≥50</td>
<td>37</td>
<td>27</td>
<td></td>
<td>31</td>
<td>P &lt; 0.05</td>
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<td>Differentiation</td>
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<tr>
<td>Well</td>
<td>21</td>
<td>14</td>
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<td>Moderately</td>
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<td>17</td>
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<td>Poorly</td>
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<td>I–II</td>
<td>35</td>
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<td>19</td>
<td></td>
<td>19</td>
<td>P &lt; 0.01</td>
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<td>Lymph node metastasis</td>
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<td>22</td>
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<td>Yes</td>
<td>33</td>
<td>28</td>
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<td>31</td>
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*aWell versus moderately.  
*bWell versus poorly.

Discussion

Though the incidence of gastric cancer has recently decreased in the USA and Western European countries, it is still a major cause of cancerous death in many countries, especially in Eastern Asia, Eastern Europe and Latin America (33). Many studies showed that COX-2 played a pivotal role in gastric cancer (1,11–14). Although some progress has been made, the molecular mechanisms involved in the carcinogenesis and development of COX-2 in gastric cancer are still not fully elucidated. It is a new approach to use the comparative proteomics technique to find the molecules regulated by COX-2, though the mechanisms of COX-2 in gastric cancer have been studied by a variety of ways. In the present study, we successfully constructed the models of gastric cancer cells transfected with COX-2siRNA and firstly investigate the COX-2-regulated proteins in gastric cancer by using the comparative proteomics approach. Identification of 14 significantly differentially expressed proteins was achieved by 2-DE and MALDI-TOF MS. The functions of some proteins are involved in proliferation, p53 activity inhibitor, protein transportation, chaperones and regulation of cell cycle, regulation of cell motility, regulation of cell development of cancer as was reported (34–36). Molecular mechanisms of COX-2-derived prostaglandins in tumorigenesis included proliferation, motility, angiogenesis, invasion, metastasis, apoptosis, immunity and surveillance (17,37–44). Its amount in vivo depends on its relative speed of synthesis and degradation. In this process, synthetase and catabolic enzyme played crucial roles (27,45,46). COX-2, a key enzyme that participates in prostaglandin synthesis, was highly expressed in the course of carcinogenesis and progression of gastric cancer. It can stimulate tumor progression by upregulating prostaglandins. However, if abnormally elevated prostaglandins were simultaneously degraded, its expression would still remain normal in vivo. 15-PGDH, as the catabolic enzyme for prostaglandins, acts as a physiological antagonist against the function of COX-2, thus keeping the amount of prostaglandins in balance. In abstracto, we logically presume the expression of 15-PGDH should be upregulated to degrade the redundant prostaglandins if the amount of prostaglandins were abnormally elevated. However, in fact, 15-PGDH was found decreased or even absent in various kinds of cancers (20,25,27,29,30). Ding et al. (28) detected 15-PGDH expression in 19 pairs of non-small cell lung cancer tissues and adjacent non-tumor tissues. Two-fold decrease was found in 100% of the cancer tissues whereas 10-fold decrease was found in 61% of the cancer tissues compared with the corresponding normal-tumor tissues. After being injected with non-small cell lung cancer A549 cells expressing wild-type 15-PGDH, mice displayed a significant decrease in tumor growth compared with the control groups. Yan et al. (30) detected 15-PGDH expression in 21 cases of normal tissues from healthy volunteers and 38 cases of colon cancer tissues. The results showed that, when compared with the normal tissues, 15-PGDH was barely expressed in colon cancer tissues, with at least 17-fold decrease. When 15-PGDH expression was reversed, tumor proliferation significantly slowed down in nude mice. Furthermore, 15-PGDH was found significantly decreased in several colon cancer cell lines. The same results were also found in breast cancer, medullary thyroid cancer and prostate cancer (20,25,29). Moreover, in lung cancer, Tong et al. (47) found that after COX-2 was increased by inducing agents, such as interleukin 1β, tumor necrosis factor-α or phorbol ester, 15-PGDH was significantly downregulated. Meanwhile, adenosine-mediated restoration of COX-2 rather than COX-1 could inhibit 15-PGDH expression. Additionally, 15-PGDH was significantly increased after inhibition of interleukin 1β-induced COX-2 expression by COX-2siRNA whereas no detectable change of 15-PGDH expression presented after knock down of COX-1 expression by COX-1 siRNA. Tong et al. concluded that overexpression of COX-2 but not COX-1 led to the decreased expression of 15-PGDH in lung cancer. However, in the researches on gastric cancer, no coincident conclusion was obtained from the extant studies (30,31). We examined paired expression of COX-2 and 15-PGDH in 55 pairs of gastric cancer tissues and corresponding adjacent non-tumor tissues. 15-PGDH expression was found significantly reduced or even absent in gastric cancer tissues compared with adjacent non-tumor tissues and its expression was related to differentiation, TNM staging and the lymph node metastasis. In our study, we also examined expression of 15-PGDH in gastric inflammatory tissues and found that its immunostaining was increased compared with gastric cancer tissues but decreased compared with the adjacent normal tissues. 15-PGDH decreased progressively from normal tissue, inflammatory tissue, to cancerous tissue, suggesting that 15-PGDH may play at least a 2-fold increase in 75% tumor tissues (Figure 3C and D). These results were consistent with the results from immunohistochemistry analysis.
an important role as a tumor suppressor considering its more decreased expression in gastric cancer. Simultaneous 15-PGDH and COX-2 staining in successive sections indicated that 15-PGDH expression was negatively correlated to COX-2 expression ($P < 0.01$). Immunocytochemical and western blotting assay also revealed that 15-PGDH was upregulated in SGC7901-COX-2 siRNA cells whereas it was downregulated in SGC7901-COX-2 cells, further confirming that modulating COX-2 expression could significantly influence 15-PGDH expression and that the decrease of 15-PGDH expression in gastric cancer was regulated by COX-2. The results was in accordance with the finding in lung cancer that 15-PGDH was regulated by COX-2 but not COX-1 (47). Thus, we delineate that COX-2, highly expressed in gastric cancer, not only stimulates the synthesis of prostaglandins but also suppresses the degradation of prostaglandins by down-regulating the expression of 15-PGDH. Due to both disorders of synthesis and degradation, abhorrent magnified amount of prostaglandins accumulates in vivo, further leading to carcinogenesis and progression of gastric cancer.

In conclusion, 15-PGDH was significantly downregulated in gastric cancer, with its expression negatively related to the differentiation, TNM staging and lymph node metastasis of gastric cancer, which suggested that it might act as a tumor suppressor in gastric cancer. Besides, its expression was also negatively correlated to COX-2 expression. Furthermore, we confirmed that 15-PGDH was regulated by COX-2 by immunohistochemistry and western blotting. However, the underlying mechanisms are still unknown and we are therefore currently studying them. These findings may have important clinical implications and are valuable for further study of the mechanisms of COX-2-related gastric cancer. Besides, proteomics provides a novel method for detection of COX-2-regulating proteins. Future studies on the other 13 identified proteins may yield novel clues for elucidating the mechanisms of carcinogenesis and progression of gastric cancer.

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### References


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