15-PGDH is reduced and induces apoptosis and cell cycle arrest in gastric carcinoma

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Abstract

AIM: To investigate the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in human gastric cancer and its mechanism in apoptosis and cell cycle arrest.

METHODS: Expression of 15-PGDH mRNA and protein was examined by immunohistochemistry, immunocytochemistry, reverse transcriptase polymerase chain reaction (RT-PCR) and Western blotting in tissue from human gastric cancer, gastric precancerous state (gastric polyps and atrophic gastritis), normal stomach, and gastric cancer cell lines. The relationship between gastric cancer, gastric precancerous state and 15-PGDH expression was determined. The association between expression of 15-PGDH and various clinicopathological parameters in gastric cancer was evaluated. Human gastric cancer cell line SGC-7901 was transfected with 15-PGDH expression plasmids. The effect of 15-PGDH on cell cycle was examined by flow cytometry. The effect of 15-PGDH on apoptosis was examined by transmission electron microscopy, flow cytometry and transferase mediated nick end labeling (TUNEL) assay. Expression of cell cycle (p21, p27, p16 and p53) and antiapoptotic genes (BCL-2, BCL-XL, BAX and BAX) genes was analyzed by RT-PCR.

RESULTS: Expression of 15-PGDH mRNA and protein in human gastric cancer tissues was significantly lower than in normal gastric tissues (P < 0.01). Expression in human gastric cancer cell lines MKN-28 and MKN-45 was reduced, and absent in SGC-7901 cells (P < 0.05). Reduction of 15-PGDH expression was also found in precancerous tissues, such as gastric polyps and atrophic gastritis (P < 0.01). There was a significant difference in expression of 15-PGDH among various gastric cancer pathological types (P < 0.05), with or without distant metastasis (P < 0.05) and different TNM stage (P < 0.01). Flow cytometry demonstrated a significant increase in apoptotic cells in SGC-7901 cells transfected with pcDNA3/15-PGDH plasmid for 24 h and 48 h (P < 0.01), and an increased fraction of sub-G1 phase after transfection (P < 0.05). TUNEL assay showed an increased apoptotic index in cells overexpressing 15-PGDH (P < 0.01). After transfection, expression of proapoptotic genes, such as BAK (P < 0.05), BAX and p53 (P < 0.01) was increased. Expression of antiapoptotic genes was decreased, such as Survivin, BCL-2 and BCL-XL (P < 0.01). Expression of cyclin-dependent kinase inhibitors p21 and p16 (P < 0.01) was significantly upregulated in cells overexpressing 15-PGDH.

CONCLUSION: Reduction of 15-PGDH is associated with carcinogenesis and development of gastric carcinoma. 15-PGDH induces apoptosis and cell cycle arrest in SGC-7901 cells.
Introduction

Gastric carcinoma is one of the most common malignant tumors in humans and continues to be a major unresolved health problem. New approaches for the management of gastric cancer are needed. NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is the key enzyme responsible for the biological inactivation of prostaglandins (PGs) and related eicosanoids. It catalyzes the oxidation of the 15(S)-hydroxyl group of PGs and lipoxins. The products, 15-ko-metabolites, exhibit greatly reduced biological activities[1]. 15-PGDH is widely distributed in various mammalian tissues such as lung, breast, prostate, placenta and gut. The stomach is one of the most active tissues expressing 15-PGDH[2]. Recent studies[3-14] have shown a reduction of 15-PGDH in some cancers, such as colorectal, breast, prostate and lung. Some studies[15-17] have revealed that 15-PGDH may have tumor-suppressive properties. Recently, some studies[18-20] have indicated that 15-PGDH is downregulated in gastric cancer and is a suppressor of human gastric cancer. It provides a new target for the chemoprevention of gastric cancer and is a suppressor of human gastric cancer. Our results suggest the use of 15-PGDH in chemoprevention and treatment of gastric cancer.

Materials and Methods

Human gastric specimens

Human gastric carcinoma specimens (n = 30) were obtained from surgical resections, with the approval of the Shanghai First People’s Hospital Ethics Committee. The specimens were frozen and stored in liquid nitrogen and 10% formaldehyde solution. Each tumor sample was matched with adjacent tissues (3 cm and 6 cm from the border of tumor) collected during the process. Other gastric tissues, including normal gastric tissues (n = 10), gastric polyps (n = 10) and chronic atrophic gastritis (n = 10), were obtained from gastroscopic biopsy and stored in liquid nitrogen and 10% formaldehyde solution. Specimens were dissected macroscopically by trained pathologists.

Cell culture

Human gastric carcinoma cell lines MKN-45, MKN-28 and SGC-7901 (obtained from Shanghai Institute of Biochemistry and Cell Biology) were maintained in RPMI-1640 ( Gibco, United States) medium supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin in a 5% CO2 atmosphere at 37℃. These cells were plated in six-well plates at about 2 × 104 cells/well in duplicate, and grown for 24 h before transfection.

Expression of wild-type 15-PGDH

The mammalian expression vector pcDNA3 containing the cDNA of the wild-type 15-PGDH and pcDNA3 expression vector were donated by Dr. Tai HH (Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, United States). Both pcDNA3/15-PGDH and pcDNA3 (200 ng) plasmids were transfected into SGC-7901 cells by Lipofectamine 2000 reagent for 24 h and 48 h, according to the manufacturer’s directions. Expression of the wild-type 15-PGDH mRNA and protein was monitored by reverse transcription polymerase chain reaction (RT-PCR), cellular immunohistochemistry and Western blotting.

Immunohistochemistry and immunocytochemistry

Paraffin-embedded tissue sections (3 μm) were dried, deparaffinized, and rehydrated. Endogenous peroxidase was blocked with 3% hydrogen peroxide in ion-free water for 30 min. After nonspecific binding sites, tissue slides were blocked with 10% goat serum. Cellular slides were treated by 4% paraformaldehyde for 30 min. Both kinds of slides were incubated at 4℃ overnight with a 1:50 dilution of rabbit polyclonal 15-PGDH antibody (Cayman, United States), followed by a 30-min incubation in horseradish peroxidase (HRP)-conjugated sheep anti-rabbit IgG (Changdao, China), rinsed with PBS, developed with the DAB kit (DakoCytomation, United States), and then counterstained with haematoxylin. Each slide

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Reverse transcriptase polymerase chain reaction analysis

Total RNA of tissues and gastric cancer cells was extracted with TRIzol (Invitrogen, United States) following the manufacturer’s instructions. cDNA was synthesized from 2 μg total RNA using the M-MLV RT-PCR kit (Promega, United States) in a 20 μL volume, according to the manufacturer’s instructions. Two μL of cDNA, 2 μL each primer (50 pmol/L), 1 μL dNTP mix (10 mmol/L) and 1 μL Taq DNA polymerase (Sangon, China) were used for PCR analysis. The PCR amplification cycles consisted of denaturation at 94 ℃ for 5 min, 35 cycles of denaturation at 94 ℃ for 60 s, annealing for 60 s, extension at 72 ℃ for 60 s, and final elongation at 72 ℃ for 10 min. The PCR products were separated on a 1.5% agarose gel, stained with 0.5 mg/mL ethidium bromide, and visualized by UV light. Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase and shown as the ratio of absorbance values. The primer sequences and annealing temperature are listed in Table 1.

Western blotting

Tissues and gastric cancer cells were lysed with lysis buffer containing 0.5% NP-40, 40 mmol/L Tris-HCl (pH 8.0), 120 mmol/L NaCl, and a protease cocktail inhibitor (Complete Mini; Pierce, Rockford, Ill., United States). Samples (40 μg protein per lane) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then blotted onto polyvinylidene difluoride membranes. Membranes were blocked for 2 h at room temperature with 5% skimmed milk and then probed with 1:200 dilution of rabbit polyclonal 15-PGDH antibody overnight at 4 ℃. Membranes were washed and incubated for 1 h at room temperature with anti-rabbit IgG-HRP. Results were visualized by ECL chemiluminescence detection kit (Kangcheng, China). Protein expression was normalized to ACTIN.

Cell cycle analysis and apoptosis assays

The effect of 15-PGDH on the cell cycle and apoptosis in SGC-7901 cells was analyzed by flow cytometry. Cells floating in medium combined with the adherent layer were trypsinized and fixed with 2 mL citrate buffer for 1 h. Cells were then incubated with RNase A (1500 μL) and stained with propidium iodide (1500 μL). Samples were immediately analyzed by flow cytometry for cell cycle and apoptosis assays. Cells were observed under transmission electron microscopy (TEM) at Shanghai Medical College of Fudan University. The number of apoptotic cells was counted per 100 cells. Terminal deoxynucleotidyl transferase mediated nick end labeling (TUNEL) assay, in which residue of digoxigenin-labeled dUTP was catalytically incorporated into the DNA by terminal deoxynucleotidyl transferase II, was performed according to the manufacturer’s instructions (Boster, Wuhan, China). The positive particles of DAB staining were viewed under an optical microscope. The number of apoptotic cells was counted under a microscope (400 ×) and expressed as the apoptotic index (AI = the number of apoptotic bodies/1000 cells).

15-PGDH: 15-hydroxyprostaglandin dehydrogenase.

### Table 1  Polymerase chain reaction primers

| Target genes | Primer sequence | Size (bp) | Annealing temperature (℃) |
|----------------|-----------------|-----------|--------------------------|
| GAPDH Sense | 5’-CCACCCCATGGCACAATTTCCATGGCA-3’ | 593 | 62 |
| GAPDH Antisense | 5’-AACAAAGGCTTGACAATAAAT-3’ | 5’-CCACCCCATGGCACAATTTCCATGGCA-3’ | 593 | 62 |
| 15-PGDH Sense | 5’-GCTGAGTGATTAATGAGA-3’ | 285 | 55 |
| 15-PGDH Antisense | 5’-GCTGAGTGATTAATGAGA-3’ | 5’-GCTGAGTGATTAATGAGA-3’ | 285 | 55 |
| Survivin Sense | 5’-GCAGGCTCAATATCAGAGA-3’ | 320 | 58 |
| Survivin Antisense | 5’-GCAGGCTCAATATCAGAGA-3’ | 5’-GCAGGCTCAATATCAGAGA-3’ | 320 | 58 |
| BCL-2 Sense | 5’-GCTGACCAACTGTGGTCGCC-3’ | 458 | 54 |
| BCL-2 Antisense | 5’-GCTGACCAACTGTGGTCGCC-3’ | 5’-GCTGACCAACTGTGGTCGCC-3’ | 458 | 54 |
| BAX Sense | 5’-CTGACTATTTCTGACGCCGT-3’ | 289 | 54 |
| BAX Antisense | 5’-CTGACTATTTCTGACGCCGT-3’ | 5’-CTGACTATTTCTGACGCCGT-3’ | 289 | 54 |
| BCL-Xl Sense | 5’-TTGGCAATAGCTGTTGT-3’ | 765 | 54 |
| BCL-Xl Antisense | 5’-TTGGCAATAGCTGTTGT-3’ | 5’-TTGGCAATAGCTGTTGT-3’ | 765 | 54 |
| BAK Sense | 5’-TGAGAGTGGATGGTCAGTG-3’ | 642 | 54 |
| BAK Antisense | 5’-TGAGAGTGGATGGTCAGTG-3’ | 5’-TGAGAGTGGATGGTCAGTG-3’ | 642 | 54 |
| p53 Sense | 5’-CCCTCCAAGAAAACCTACCA-3’ | 371 | 59 |
| p53 Antisense | 5’-CCCTCCAAGAAAACCTACCA-3’ | 5’-CCCTCCAAGAAAACCTACCA-3’ | 371 | 59 |
| p21 Sense | 5’-CAGGGCCAGCAAGAGGACAG-3’ | 335 | 63 |
| p21 Antisense | 5’-CAGGGCCAGCAAGAGGACAG-3’ | 5’-CAGGGCCAGCAAGAGGACAG-3’ | 335 | 63 |
| p27 Sense | 5’-GCGCAGATAGATCAGGACAG-3’ | 395 | 58 |
| p27 Antisense | 5’-AGTCCAACGCTGCGAGCACC-3’ | 5’-GCGCAGATAGATCAGGACAG-3’ | 395 | 58 |
| p16 Sense | 5’-GGGCGGCGACAGAGGACAG-3’ | 357 | 59 |
| p16 Antisense | 5’-GGGCGGCGACAGAGGACAG-3’ | 5’-GGGCGGCGACAGAGGACAG-3’ | 357 | 59 |
Statistical analysis
Quantitative results were expressed as mean ± SD. Statistical analysis was assessed by Student’s t test (between two groups) or Student-Newman-Keuls test (among three or more groups), with SAS version 8.02 software. P < 0.05 was considered statistically significant.

RESULTS
Downregulation of 15-PGDH expression in gastric cancer, paracancerous and precancerous tissues and gastric cancer cell lines
Immunohistochemistry analysis confirmed that 15-PGDH protein was expressed mainly in the cytoplasm of epithelial, inflammatory and gastric cancer cells in the lamina propria. Of the 30 gastric cancer cases, 15-PGDH expression was undetectable in 10 tumors (33.3%). Immunohistochemistry score of 15-PGDH was decreased in gastric cancer, paracancerous tissues 3 cm and 6 cm from the tumor, gastric polyps and atrophic gastritis compared with normal gastric tissues. Immunocytochemical analysis showed that expression of 15-PGDH in various differentiated gastric cell lines was dissimilar. Poorly differentiated gastric cell line SGC-7901 displayed no 15-PGDH, whereas MKN-28 and MKN-45 displayed little 15-PGDH (Figure 1).

RT-PCR analysis showed that expression of 15-PGDH mRNA in gastric cancer, paracancerous tissues, gastric polyps and atrophic gastritis was significantly lower than in normal gastric tissues. We also found loss of 15-PGDH in nine tumors (30%). Expression of 15-PGDH was absent in SGC-7901 cells, and significantly decreased in MKN-45 and MKN-28 cells (Figure 2).

Western blotting demonstrated that 15-PGDH protein expression was absent in nine of 30 gastric cancer cases (30%), and an average 5.7- and 8.3-fold less 15-PGDH expression was found in cancer tissues compared with paracancerous tissues at 3 cm and 6 cm from the tumor. There was a twofold reduction in gastric polyps and 2.1-fold reduction in atrophic gastritis tissues compared with normal gastric mucosa. Expression of 15-PGDH

Figure 1 Immunohistochemistry image of 15-hydroxyprostaglandin dehydrogenase (400 ×). A: Gastric cancer (IHC Score 1.20 ± 1.13, P < 0.01 vs B, C and F); B and C: Paracancerous tissue 3 cm (6.83 ± 2.78, P < 0.01 vs C and F) and 6 cm (10.20 ± 1.92) distant from tumor; D: Gastric polyps (6.00 ± 2.74, P < 0.01 vs F); E: Atrophic gastritis (5.14 ± 1.57, P < 0.05 vs F); F: Normal gastric tissues (11.00 ± 1.63). G: MKN-28 (3.31 ± 0.92, P < 0.05 vs H and I); H: MKN-45 (1.29 ± 0.48); I: SGC-7901 (absent).
protein in gastric cancer cells was the same as shown by RT-PCR (Figure 3).

**Relationship between expression of 15-PGDH and clinicopathological parameters in gastric cancer**

Expression of 15-PGDH protein was significantly different among the various gastric cancer pathological types \((P < 0.05)\). Reduction of 15-PGDH was more distinct in gastric cancer with distant metastasis than in tumor without distant metastasis \((P < 0.05\) at protein level, \(P < 0.01\) at mRNA level). There was also a significant difference in expression of 15-PGDH among tumors of different TNM stage \((P < 0.01\) at both protein and mRNA level). More reduced 15-PGDH expression was associated with worse TNM stage (Table 2).

**Over-expression of 15-PGDH induced cell cycle arrest and apoptosis in SGC-7901 cells**

After transfection by pcDNA3/15-PGDH plasmid, SGC-7901 cells were induced to overexpress 15-PGDH (Figure 4). At the same time, an increased fraction of sub-G1 phase \((57.21\% \pm 0.53\%\) for 24 h transfection and \(57.22\% \pm 2.85\%\) for 48 h transfection, \(P < 0.05\)) was found by flow cytometry (Figure 5). It showed that 15-PGDH promoted cell cycle arrest in the sub-G1 phase. To assess the effect of 15-PGDH on induction of cell apoptosis in gastric cancer, we observed SGC-7901 cells under TEM and by flow cytometry, and then performed a TUNEL assay. Under TEM, nuclear and cytoplasmic shrinkage, condensation and margination of chromatin against the nuclear membrane, and formation of apoptotic bodies were observed in SGC-7901 cells that overexpressed 15-PGDH (Figure 6). The proportion of apoptotic cells was significantly increased after transfection for 24 h and 48 h \((12.33\% \pm 1.15\%\) and \(25.00\% \pm 1.00\% \pm 3.33\% \pm 0.58\%, \(P < 0.01\))\), which was further confirmed by TUNEL assay \((A1: 25.27\% \pm 1.19\% and 48.37\% \pm 2.67\% \pm 6.50\% \pm 0.30\%, \(P < 0.01\))\) (Figure 7). It indicated that cell cycle arrest and increased apoptosis was one mechanism of cancer suppression of 15-PGDH in SGC-7901 cells.

Expression of genes associated with the cell cycle
(p21, p27, p16 and p53) and apoptosis (Survivin, BCL-2, BCL-X, BAK and BAX) was determined by RT-PCR in SGC-7901 cells transfected with pcDNA3/15-PGDH plasmids. p21 (1.75 ± 0.51 for 24 h transfection and 1.76 ± 0.52 for 48 h transfection vs 0.46 ± 0.06 SGC-7901, P < 0.01), p16 (0.33 ± 0.12 and 0.32 ± 0.17 vs absence, P < 0.01) and p53 genes (0.19 ± 0.04 and 0.19 ± 0.06 vs 0.08 ± 0.02, P < 0.01) were significantly upregulated in cells treated with 15-PGDH for 24 h group and 48 h, whereas the level of p27 mRNA did not change (P > 0.05). Expression of the proapoptotic genes, such as BAK (0.92 ± 0.14 and 1.04 ± 0.27 vs 0.52 ± 0.24, P < 0.05) and BAX (1.73 ± 0.17 and 1.72 ± 0.07 vs 1.14 ± 0.11, P < 0.01) was significantly increased. The antiapoptotic genes, such as Survivin (0.14 ± 0.06 and 0.13 ± 0.02 vs 0.34 ± 0.06, P < 0.01), BCL-2 (0.02 ± 0.01 and 0.02 ± 0.01 vs 0.08 ± 0.03, P < 0.01) and BCL-X (0.63 ± 0.11 and 0.63 ± 0.08 vs 1.12 ± 0.08, P < 0.01), were significantly down-regulated (Figure 8).

**DISCUSSION**

These findings demonstrate that the reduction of 15-PGDH is related to occurrence and development of gastric cancer. The expression of 15-PGDH and clinicopathological parameters in gastric cancer is shown in Table 2. A flow cytometry result of gastric cancer cell cycle is shown in Figure 5.
gastric cancer in humans. 15-PGDH also induces cell cycle arrest and apoptosis in gastric cancer cells. It may be a suppressor of gastric cancer through these two pathways. 15-PGDH catalyzes the oxidation of the 15(S)-hydroxyl group of PGs and lipoxins. 15-PGDH is one of the target genes. Some cytokines, factors and cell signaling pathways affect carcinogenesis and tumor progression through 15-PGDH. It shows that epidermal growth factor (EGF) and EGF receptor tyrosine kinase inhibitors, histone deacetylase inhibitors, transforming growth factor-β (TGF-β) [18], hepatocyte nuclear factor 3β [17], interleukin (IL)-4 [19], tumor necrosis factor α [20], IL-1β [21], peroxisome proliferator-activated receptor γ ligands [22], hepatocyte growth factor receptor, Met [23], bile acids [24] adjust tumor growth through 15-PGDH. Recent studies have shown an obvious reduction of 15-PGDH in some cancers, for example, colorectal, breast, prostate and lung [25]. It also has been reported that 10%-80% of gastric cancer exhibits downregulation of 15-PGDH expression [26,27]. There was a significant reduction in gastric cancer tissues and cell lines examined. There was also a significant reduction of 15-PGDH expression in paracancerous and precancerous tissues, for example, gastric polyps and atrophic gastritis. Downregulation of 15-PGDH expression was positively correlated with differentiation in gastric cancer tissues, distant metastasis and different TNM stages of gastric cancer. This result is similar to that in previous studies. It has also been reported that expression of 15-PGDH is reduced and associated with tumor differentiation, lymph node metastasis, clinical stage [28,29] and prognosis [30] in gastric cancer. We verified the relationship between differentiation of gastric cancer cells and 15-PGDH expression in vitro. We showed that poorer differentiation in carcinoma was associated with lower 15-PGDH expression. Taken together, reduction of 15-PGDH is related to the carcinogenesis and development of gastric cancer. Evaluation of 15-PGDH expression in tumor and precancerous tissues is a useful diagnostic or prognostic marker for gastric carcinoma.

After determining the relationship between 15-PGDH expression and gastric cancer, we suggest that reduction of 15-PGDH promotes occurrence and development of gastric cancer and that it is an inhibitor of human gastric cancer. Some studies have already demonstrated that 15-PGDH suppresses some tumors. Overexpression of 15-PGDH by transfection with plasmid or adenovirus vectors encoding 15-PGDH reduces occurrence and growth of tumor [3-5,8-10], whereas silencing of 15-PGDH using siRNA enhances cell proliferation and growth of cancer [10]. 15-PGDH gene knockout increases the colon tumor incidence in the APC+/Min mouse model [31]. The antitumor effect in human gastric cancer has only been shown in one study [32]. However the mechanism is still not clear.

The mechanism of the antitumor effect of 15-PGDH can be explained by the following hypothesis. 15-PGDH substantially inhibits production of PGE2 and changes the microenvironment to suppress tumor formation by Ras gene [9]; controls growth of tumor by regulation of cyclooxygenase-2 [21]; suppresses synthesis, secretion and

![Figure 6](Image 57x622 to 150x763) Transmission electron microscopy of gastric cancer cell apoptosis (5000 ×). A: Normal configuration of SGC-7901 cell; B: Normal configuration of cell in pcDNA3 24 h group; C: Apoptotic cell in pcDNA3/15-PGDH 24 h group; D: Normal configuration of cell in pcDNA3 48 h group; E: Apoptotic cell in pcDNA3/15-PGDH 48 h group.

![Figure 7](Image 153x622 to 247x763) Induction of apoptosis in SGC-7901 cell line. There was a significant apoptosis in erlotinib pcDNA3/15-hydroxyprostaglandin dehydrogenase (15-PGDH) 24 h and 48 h groups in transmission electronmicroscopy, flow cytometry analysis and TUNEL assay. *P < 0.01 vs SGC-7901, pc DNA3 24 h and 48 h group; **P < 0.01 vs SGC-7901, pcDNA3/15-PGDH 24 h group, pcDNA3 24 h and 48 h group.
activation of matrix metalloproteinase-2, inhibits cell adhesion to extracellular matrix and reduces CD44 expression, which contributes to the inhibition of the growth, invasion and metastasis of cancer cells; reduces expression of antiapoptotic protein Bcl-2, which indicates a role for Bcl-2 in mediating or triggering the event of apoptosis; inhibits endothelial cell proliferation by 15-oxo-5,8,11,13-(Z,Z,Z,E)-eicosatetraenoic acid, a metabolite of 15-PGDH, suppressing DNA synthesis and implicating a potential antiangiogenic role; and attenuates tumor-induced immune suppression and substantially reduces the secretion of immunosuppressive mediators and cytokines such as PGE2, IL-10, IL-13 and IL-6 to regulate the local antitumor immune response.

Our present research showed that apoptosis occurred in gastric cancer cells overexpressing 15-PGDH. In SGC-7901 cells transfected with pcDNA3/15-PGDH plasmid, we found by TEM nuclear and cytoplasmic shrinkage, condensation and margination of chromatin against the nuclear membrane, and formation of apoptotic bodies. Flow cytometric analysis and TUNEL assay showed that the proportion of apoptotic cells was increased by 15-PGDH. Overexpression of this enzyme induces apoptosis in lung cancer cell line A549. When A549 cells overexpress 15-PGDH by transfection with Ad-15-PGDH, they become apoptotic, as shown by DNA fragmentation, activation of procaspase-3 and cleavage of poly ADP ribose polymerase.

Furthermore, we analyzed genes associated with apoptosis. There was a reduction in expression of antiapoptotic genes (Survivin, BCL-2 members and p53) and increased expression of proapoptotic genes (BAK, BAX and p53). As we know, Survivin, BCL-2 members and p53 genes are crucial regulators of apoptotic cell death. BCL-2 prevents the release of apoptosis-inducing factor and cytochrome c from the mitochondria, which is assumed to be a key event during apoptosis. Overexpression of 15-PGDH regulates expression of Survivin, BCL-2 members and p53, indicating a role in mediating or trig-
15-Hydroxyprostaglandin dehydrogenase (15-PGDH) suppresses gastric cancer

**Gerkinget apoptosis. The results are inconsistent with previous findings, in which apoptosis induced by 15-PGDH was independent of the p53 pathway. 15-PGDH only decreases expression of antiapoptotic protein BCL-2 in lung cancer.** The mechanism of apoptosis varies in different tumors. In gastric cancer, 15-PGDH induces apoptosis by Survivin, BCL-2 and the p53 pathway.

In our study, we also observed cell cycle arrest in SGC-7901 cells that overexpressed 15-PGDH. We showed an increased accumulation of cells in the sub-G1 phase compared with the control group, and upregulated expression of p21, p16 and p53 without altering p27 expression. p16, p21 and p27 all belong to the cyclin-dependent kinase (CDK) inhibitors. The product of p16 gene is an inhibitor of CDK4. Its function is to cyclin E and CDK2 complexes. p27 functions as a negative regulator of G1 progression and is a possible mediator of TGF-β-induced G1 phase arrest. p53 is known as a suppressor gene that can adjust the cell cycle.

In conclusion, our study provides evidence that loss or reduction of 15-PGDH is related to human gastric cancer. 15-PGDH induces cell cycle arrest and apoptosis of gastric cancer cells in vitro, and it may be the mechanism by which it suppresses human gastric cancer and other tumors. Further research is needed to establish the role of 15-PGDH as a target for treatment and chemoprevention of gastric cancer.

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