Anticancer activity of *PDSS2*, prenyl diphosphate synthase, subunit 2, in gastric cancer tissue and the SGC7901 cell line

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The aim of this study was to assess whether *PDSS2* (prenyl diphosphate synthase, subunit 2), a candidate tumor suppressor protein, has a potential anticancer role in human gastric cancer tissue and the SGC7901 gastric cell line. A *PDSS2* eukaryotic expression vector was constructed and introduced into SGC7901 cells. The relationship between *PDSS2* expression and cell proliferation, cell cycle distribution, and apoptosis in tumor cells was analyzed by RT-PCR, western blotting, the MTT colorimetric assay, flow cytometry, and immunohistochemistry. Increased exogenous *PDSS2* expression *in vitro* is associated with decreased cellular proliferation of the gastric cancer cell line SGC7901. PDSS2 also induced apoptosis in SGC7901 cells by causing cell cycle arrest in the G_0/G_1 phase. Moreover, a significantly low expression level of PDSS2 protein was found in gastric cancer. Decreased or absent expression of PDSS2 was showed in the gastric tumor biopsy samples analyzed, correlating with cancer differentiation. PDSS2 has potent anticancer activity in gastric cancer tissues and the SGC7901 cell line and is possibly involved in apoptosis in SGC7901 cells. *Anti-Cancer Drugs* 20:141–148 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Gastric cancer is the second most common cancer and cause of cancer deaths throughout the world [1]. It is well known that gastric cancer is a complex disease that involves the accumulation of both genetic and epigenetic alterations of numerous genes [2,3]. Chromosomal abnormality and genetic defects play important roles in the occurrence and development of gastric cancer. Several mechanisms are known to activate or inactivate genes, including mutation, gene loss of heterozygosity (LOH), homozygous deletion, and methylation. The precise mechanism of each of these genetic alterations in the multistep pathway of gastric cancer development is, however, poorly understood. LOH occurs more frequently than gene mutations in tumors examined from all sites. Cytogenetic and molecular genetic analyses have identified the long arm of chromosome 6 as a frequent target for gastric cancer [4]. Detailed mapping of LOH revealed at least two frequently deleted genomic regions on chromosome 6q16.3-6q23 and 6q26-27 which could harbor one or more tumor suppressor genes (TSGs) [5,6]. Our previous study also found frequent LOH of loci in the 6q16.3-6q23 region, and indicated that loss of this region was also a frequent event in gastric cancers in Chinese populations [7]. On the basis of this finding and the completion of the human genome and gene maps, other likely candidate genes on chromosome 6q have appeared. Here, we describe the gene expression analysis of a new candidate gene, *PDSS2* (prenyl diphosphate synthase, subunit 2), in gastric tumors. *PDSS2* was found in the frequent LOH region of 6q16.3-6q21. The gene product is a candidate tumor suppressor protein, which was hypothesized as a target for 6q deletion and rearrangement in a melanoma cell line by Guan *et al.* [8]. To date, only two research groups have reported studies focused on the effect of *PDSS2* prenyl diphosphate synthase activity [9] and mutations of *PDSS2* in patients with CoQ10 deficiency [10,11]. The role that *PDSS2* plays in gastric tumorigenesis, however, has not been reported to our knowledge.

To identify the anticancer function of *PDSS2*, we constructed a full-length *PDSS2* cDNA eukaryotic expression vector and transfected gastric cancer SGC7901 cells with this vector. We found that the *PDSS2* gene can suppress growth of the gastric tumor cell line SGC7901 and induce apoptosis in SGC7901 cells. Analysis of gastric tissue samples showed significantly low frequencies of PDSS2 protein in metastases and gastric cancers. We conclude that *PDSS2* exhibits some properties of tumor suppressor and suggest that the state of this gene may have significant prognostic potential in assessing the progression of human gastric cancer.

Materials and methods

Cell lines and tissue samples

The SGC7901 cells derived from a human gastric cancer were used for transfection. It is a kind gift of Shaanxi ChaoYing Biotechnology Co. Ltd., China. A total of 39 gastric cancer tissues and 12 adjacent normal tissues were studied using immunohistochemistry. The

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Table 1 Oligonucleotide primers used for RT-PCR

Name	Upstream primer (5′ – 3′)	Downstream primer (5′ – 3′)	Size (base pair)
PDSS2-full	CGCGAATTCCACCA TGAACTTTCGGC	CCGCTCGAGTCA TGAAAATCTGGTCAC	1245
PDSS2-part	GGTGCCTTACTA GCAAAGAG	GCCTTGTTTCCATG GTAACG	350
GAPDH	TTACTCCTTGGAGG CCATGTGGGCC	ACTGCCACCCAGA AGACTGTGGATGG	465

GAPDH, glyceraldehyde 3-phosphate dehydrogenase, PDSS2, prenyl diphosphate synthase, subunit 2.

paraffin-embedded tissue specimens were derived surgically from patients with pathologically proven gastric carcinoma at the First Affiliated Hospital of Medical College of Xi'an Jiaotong University from 2005 to 2006. The pathologic tumor staging was determined according to the American Joint Committee on Cancer tumor–node–metastasis classification [12]. The experimental protocols were approved by the Xi'an Jiaotong University Clinical Trials Administration Committee and performed according to the guidelines for the care and use of clinical samples in Xi'an JiaoTong University, Xi'an, China.

Construction of the *PDSS2* eukaryotic expression vector

Total cellular RNA was isolated from normal gastric tissue by using TRIzol reagent. Total RNA (1–5 μg) was used to make first-strand cDNA with the First-Strand cDNA Synthesis Kit (Fermentas Inc., Maryland, USA) with an oligo-dT primer. First-strand cDNA synthesis was followed by standard PCR amplification (Invitrogen, Carlsbad, California, USA) using different primer sets (Table 1), and PCR yielded a 1245-bp full length *PDSS2* product with *Eco*RI and *Xhol* I sites at the 5′ end and 3′ end, respectively. The full-length *PDSS2* cDNA from normal gastric tissue was cloned first in a pMD-18T plasmid, and then into pcDNA3.1 to create pcDNA3.1-*PDSS2* (*Eco*RI/*Xhol* I). Rescued positive clones were analyzed by restriction enzyme analysis and sequencing (Bejing Augct Inc., Beijing, China). DNA homology searches were carried out by using the blastp and blastn programs at the NCBI server (*www.ncbi.nlm.nih.gov/blast*).

Cell culture and transfections

The SGC7901 cells derived from human gastric cancer were used and cultured in RPMI 1640 (Gibco, Grand Island, New York, USA) supplemented with 10% fetal calf serum (Gibco) and incubated at 37°C with 5% CO_2. Cells were transfected with different amounts of the pcDNA3.1-*PDSS2* expression plasmid or the pcDNA3.1 plasmid alone (1.0 μg per 4×10^5 cells, 2.5 μg per 4×10^5 cells, 4.0 μg per 4×10^5 cells) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's recommendations. Six hours posttransfection, the transfection medium was replaced by RPMI 1640 plus 10% FCS. Cells were harvested 24, 48, or 72 h posttransfection. RT-PCR and western blot analysis were carried out to determine the transfection efficiency.

In-vitro cell proliferation assay after transfection

Cells were transferred to 96-well plates (4×10^4 cells in each well) and were transfected as described above 24 h after the cells had adhered to the plates. Survival rate and the ability to proliferate were examined 24, 48, or 72 h later by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. Cells were incubated at 37°C in 50 μl of MTT (5 mg/ml) for 4 h. After the medium and MTT were removed, 200 μl of dimethyl sulfoxide was added to each well and the cells were incubated on a plate shaker for 10 min at room temperature. Absorbance at 570 nm of the mixture was measured by using a microplate enzyme-linked immunosorbent reader. Cell survival rate was calculated as the percentage of MTT inhibition as follows: percentage of survival = (mean experimental absorbance/mean control absorbance) × 100%.

Flow cytometric analysis of cell cycle distribution

Cells were harvested at 24–48 h posttransfection, fixed overnight with cold 70% ethanol and stained with 50 μl of propidium iodide (PI) solution (1 mg/ml) and 100 μl of ribonuclease (500 μg/ml). After incubation at room temperature for 30 min in the dark, cells were analyzed by flow cytometry at 488 nm for DNA synthesis and cell cycle status (FACSCaliber instrument, Becton Dickinson, with Cell Quest software, San Jose, California, USA).

Apoptosis was assessed by using an annexin V–fluorescein isothiocyanate (FITC) apoptosis detection kit (Jingmei Biotech Company Ltd, China) [13–14]. According to the manufacturer's protocol, 48 h after transfection the cells were collected and washed twice with PBS, followed by resuspension in 500 μl of staining solution containing a fluorescein isothiocyanate-conjugated annexin V antibody (5 μl) and PI, 5 μl of 250 μg/ml stock solution. After incubation for 30 min in the dark, cells were analyzed by flow cytometry. Basal apoptosis and necrosis were identically determined on untreated cells.

Staining of apoptotic cells with Hoechst 33342

Nuclei were counterstained with Hoechst 33342 (Molecular Probes, Eugene, Oregon, USA). Coverslips containing cells transfected for 48 h were fixed with 4% paraformaldehyde and washed with 0.1 mol/l of PBS (pH 7.2) before use. Fifty microliter of Hoechst 33342 (5 μg/ml) was dropped onto the coverslip and incubated with cells for 10 min in the dark. Apoptotic cells were evaluated by fluorescence microscopy.

Transmission electron microscopy

At 48 h after transfection, cells were fixed, dehydrated, embedded, and sectioned. The sections were stained

with lead citrate and uranyl acetate and observed by electron microscopy. The characteristic morphologic changes of *PDSS2* transfected cells were recoded, including chromatin condensation, plasma membrane blebbing, cell shrinkage, and fragmentation into membrane-bound bodies.

Immunohistochemistry analysis of gastric cancer tissue

For immunohistological analysis, tissue samples were fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm. Antibody to PDSS2, a newly established polyclonal antibody against PDSS2 in our laboratory, was used for the immunodetection of antigens on rehydrated sections. In brief, after blocking endogenous peroxidase activity and nonspecific protein binding, sections were incubated with anti-PDSS2, antibody (1 : 400) overnight. Sections were then incubated with biotinylated anti-rabbit immunoglobulin and peroxidase-conjugated streptavidin and developed with diaminobenzidine. Developed sections were counterstained with hematoxylin.

Statistical analysis

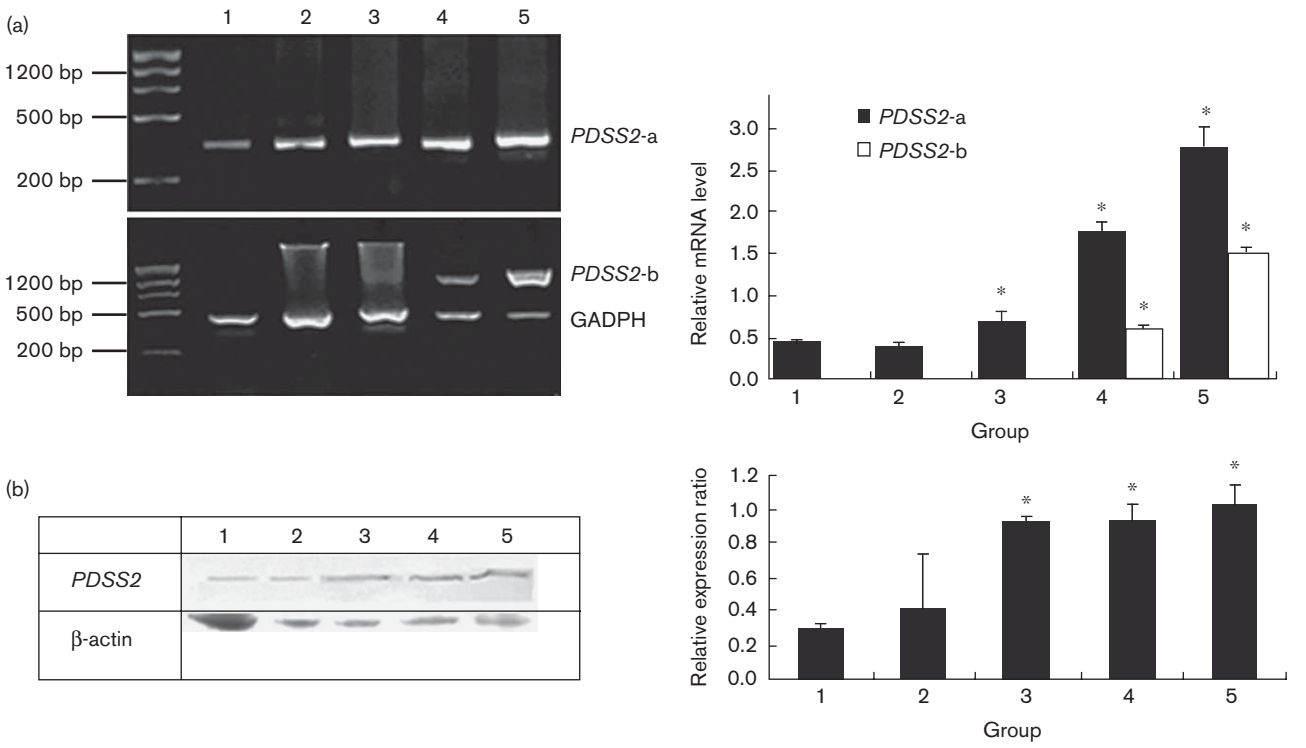
All assays were repeated three times to ensure reproducibility. The data are expressed as mean ± SD and were analyzed by SPSS13.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Significant differences were established at P value of less than 0.05.

Results

PDSS2 was highly expressed in SGC7901 cells after transfection

We obtained the full-length *PDSS2* cDNA from normal gastric tissue and cloned it into the pcDNA3.1 plasmid to generate a eukaryotic express vector. RT-PCR was used to detect a portion spanning exons 5–8 (*PDSS2*-a) and a full-length product (*PDSS2*-b) in transfected and untransfected SGC7901 cells (Fig. 1a, left). *PDSS2* mRNA levels were not changed between untransfected cells and cells transfected with empty plasmid, but *PDSS2* mRNA increased in *PDSS2* transfected cells in a dose-dependent manner 48 h after transfection (Fig. 1a, right). Western blots indicated that *PDSS2* was expressed in both

Fig. 1



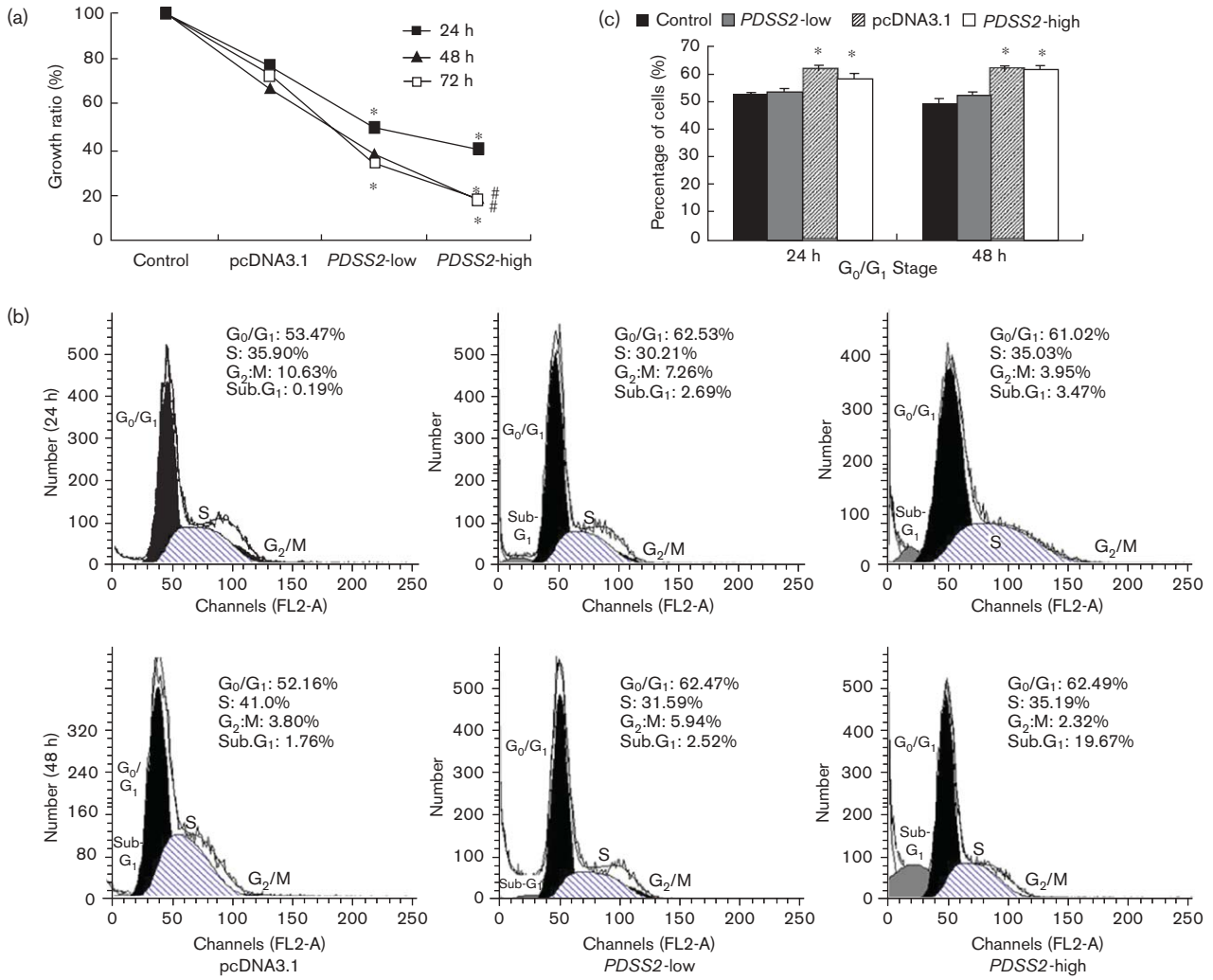
RT-PCR and western blot analysis was carried out to determine the transfection efficiency of prenyl diphosphate synthase, subunit 2 (*PDSS2*) in the human gastric cancer cell SGC7901. Group 1, untransfected control SGC7901 cells; group 2, empty pcDNA3.1 transfected cells (2.5 μg per 4×10^5 cells); groups 3, 4, and 5, pcDNA3.1-*PDSS2* transfected cells with 1.0 μg/4×10^5 cells, 2.5 μg/4×10^5 cells, 4.0 × μg/4×10^5 cells. (a) RT-PCR analysis of *PDSS2* expression at the mRNA level. Representative RT-PCR results of the *PDSS2* fragment (left). *PDSS2*-a, a partial 350-bp *PDSS2* fragment spanning exons 5–8 by using *PDSS2*-part primers; *PDSS2*-b, full-length 1245-bp *PDSS2* fragment with enzyme sites at the 5′ end and 3′ end. M, marker 3; lanes 1–5: groups 1–5. Relative mRNA levels of *PDSS2* and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (right). Analysis of semiquantitative RT-PCR was normalized according to GAPDH levels. (b) Western blot identification of *PDSS2* expression at the protein level. Representative western blot results of PDSS2 protein (left). Relative protein expression ratio of *PDSS2* in different groups (right). Analysis was normalized according to β-actin levels ($n = 3$). *$P < 0.05$ versus control.

transfected and untransfected cells, however, *PDSS2* expression in *PDSS2*-transfected cells increased significantly at 48 h posttransfection (Fig. 1b). No significant changes in cells transfected with the empty plasmid were observed. These results indicate that *PDSS2* was expressed successfully in transfected SGC7901 cells.

PDSS2 overexpression suppressed proliferation of SGC7901 cells and induced cell cycle arrest in G_0/G_1 phase

Compared with the untransfected negative control group, as shown in Fig. 2a, the growth ratios of cells transfected with the empty plasmid at 24, 48, and 72 h were 76.3, 71.7, and 73.6%, respectively. In contrast, cells transfected with a low concentration (2.5 μg per 4×10^5 cells) or high concentration of *PDSS2* (4.0 μg per 4×10^5 cells) at 24, 48, and 72 h were 49.5, 37.5, and 32.9% or 39.9, 18.1, and 17.3%, respectively. These values were significantly decreased compared with each corresponding control group at the same time. With the increase in *PDSS2* concentration and time, the cell survival rate decreased obviously and showed more and more cell growth inhibition, especially from 24 to 48 h or 72 h. However, there was no significant change between 48 and

Fig. 2



Growth suppression of gastric cancer cells by transfected prenyl diphosphate synthase, subunit 2 (*PDSS2*) expression. (a) Growth ratios of cells at different times after transfection (24, 48, and 72 h) were examined by the MTT assay. SGC7901 cells were transfected with pcDNA3.1 alone (2.5 μg per 4×10^5 cells) or vector expressing *PDSS2* (*PDSS2*-low: 2.5 μg per 4×10^5 cells, *PDSS2*-high: 4.0 μg per 4×10^5 cells). MTT reduction was then calculated to indicate cellular proliferation. Results are expressed as means ± SD of five independent determinations. Asterisks indicated significant differences from control values ($P < 0.05$). (b) Distribution of cell cycle detected by FCM induced by different concentrations of *PDSS2* in SGC7901 cells (*PDSS2*-low: 2.5 μg per 4×10^5 cells, *PDSS2*-high: 4.0 μg per 4×10^5 cells) at 24 or 48 h compared with those with cells transfected with pcDNA3.1 plasmid (2.5 μg per 4×10^5 cells). (c) The percentage of Sub-G_1, G_0/G_1, S and G_2/M cells within each cell cycle. Asterisks indicate samples with results significantly different from the control (untransfected cells) ($P < 0.05$). $^{\#}P < 0.05$, *PDSS2*-high versus *PDSS2*-low.

72 h. PI staining followed by fluorescence-activated cell sorter cell cycle distribution analysis revealed the proportions of cells in G_2/M stage in transfected *PDSS2* groups were lower than those in untransfected groups or transfected empty plasmid groups at 24 or 48 h after transfection. However, the proportion of cells in the G_0/G_1 stage increased significantly. Sub-G_1 rates of cells with low *PDSS2* concentrations were 2.69 and 2.52% at 24 and 48 h, respectively, but cells with high *PDSS2* concentrations had rates of 3.47 and 19.67%, respectively. (Fig. 2b and c).

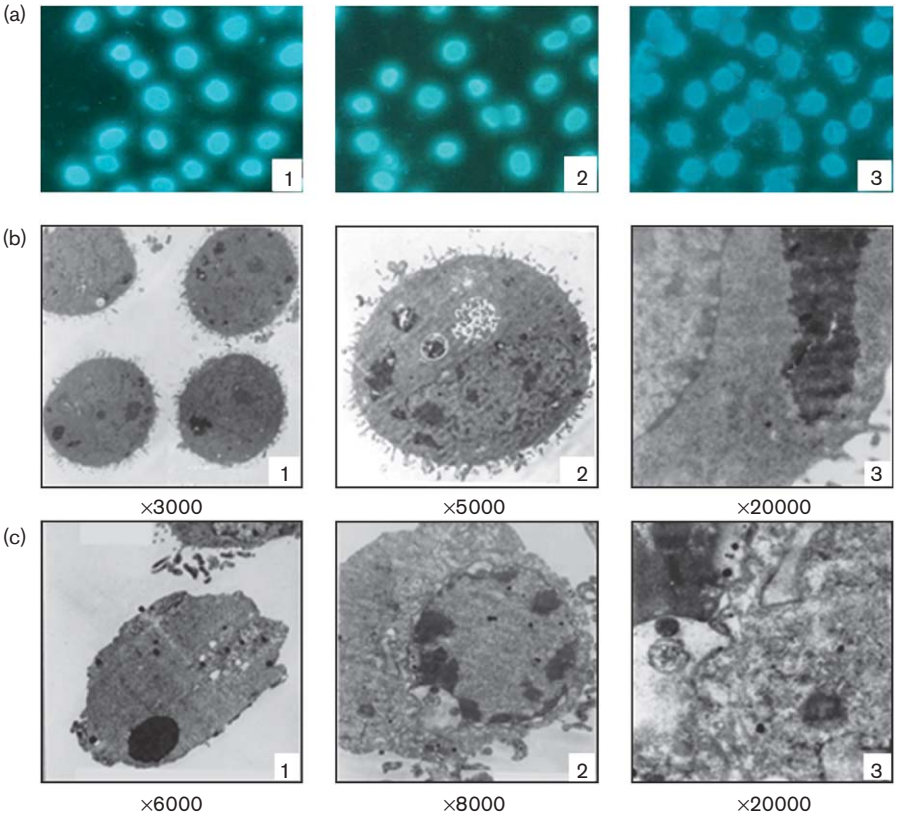
PDSS2 overexpression induced apoptosis in SGC7901 cells

In cells transfected with *PDSS2* (2.5 μg per 4×10^5 cells for 48 h), Hoechst 33342 analysis showed the presence of apoptotic cells with irregular staining of their nuclei as a result of chromatin condensation and nuclear fragmentation as well as some cells with a typical apoptotic bleb phenomenon (Fig. 3a). Transmission electron microscopy of gastric cancer SGC7901 cells transfected with *PDSS2* for 48 h also showed some morphological features of inchoate apoptosis, including cell shrinkage, chromatin condensation, plasma membrane blebbing, and formation of apoptotic bodies (Fig. 3c). The results of annexin V-PI flow cytometric detection showed that apoptosis rates at both early and late stages increased significantly in gastric cancer cells transfected with low and high concentrations of *PDSS2* ($P < 0.05$) at 48 h posttransfection (Fig. 4).

Level of PDSS2 protein expression in human gastric tumor tissue was decreased

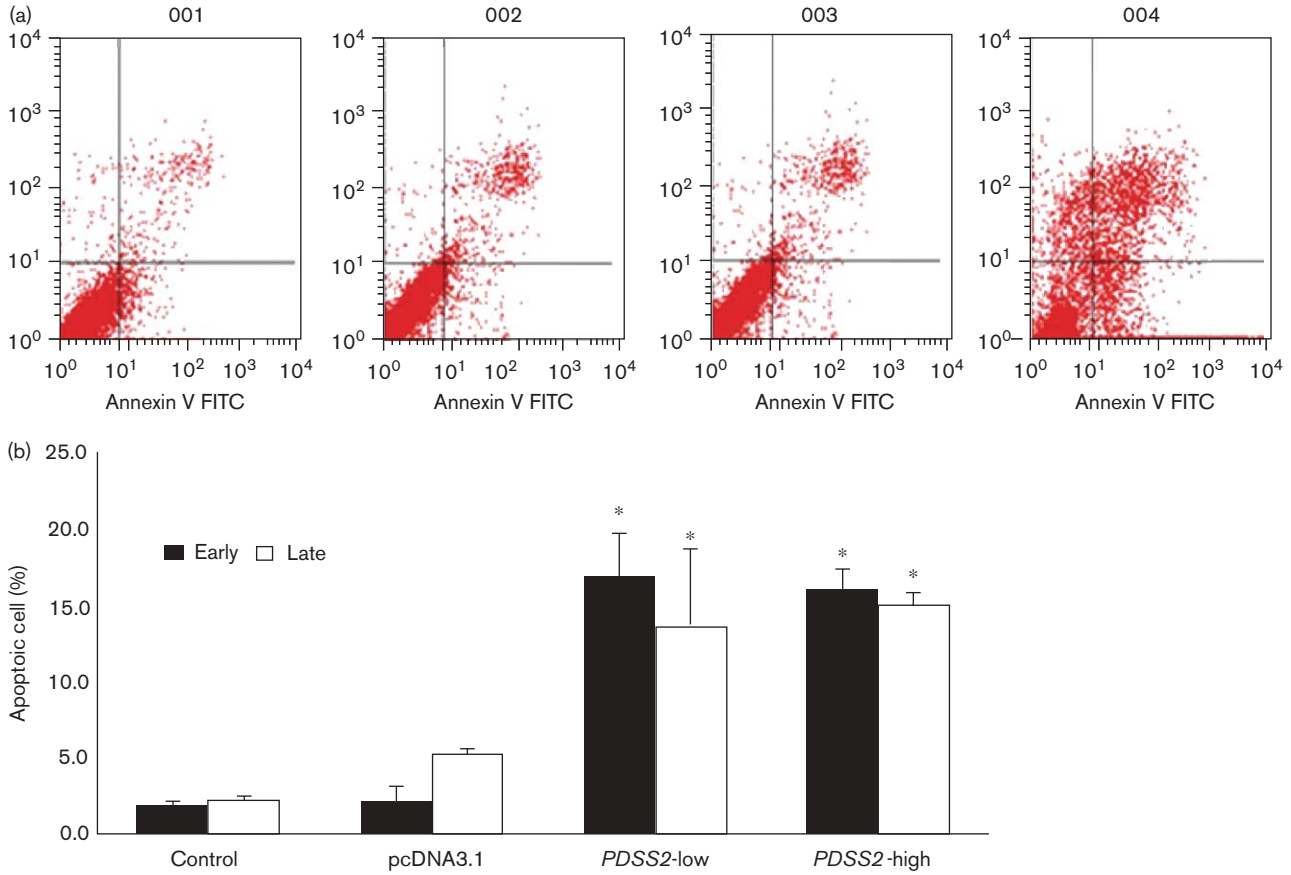
We also determined the frequency of PDSS2 protein expression in tissue specimens from different grades of human gastric tumor progression and normal gastric tissue. As shown in Fig. 5, significant reductions in *PDSS2* expression were found to occur in a stage-specific manner. *PDSS2* expression was completely lost (−) (23.1%, nine of 39) or decreased obviously (+) (61.5%,

Fig. 3



Morphological observation of apoptosis in prenyl diphosphate synthase, subunit 2 (*PDSS2*)-transfected SGC7901 cells. (a) Fluorescence photomicrographs of cells stained with Hoechst 33342 (× 200): (1) untreated SGC7901 cells showing diffusely stained intact nuclei; (2) cells transfected with pcDNA3.1 alone at 48 h posttransfection showed similar stained intact nuclei compared to control cells; (3) cells transfected with *PDSS2* (2.5 μg per 4×10^5 cells) for 48 h, apoptotic cells had condensed chromatin. (b, c) Ultrastructural changes of apoptosis in *PDSS2*-transfected cells. Untransfected control cells (b); *PDSS2*-transfected cells (2.5 μg per 4×10^5 cells) harvested 48 h posttransfection (c). Peripheral segregation and aggregation of nuclear chromatin into dense areas along the nuclear membrane was observed, and shrinkage of cells with condensation of nuclear chromatin was also readily observed in c1, compared with cells in b2. The membrane of the *PDSS2*-transfected cells had an irregular perimeter with nucleus buds (c2), and the cytoplasm contained dilated organelles such as mitochondrion and abundant vacuoles (c3).

Fig. 4



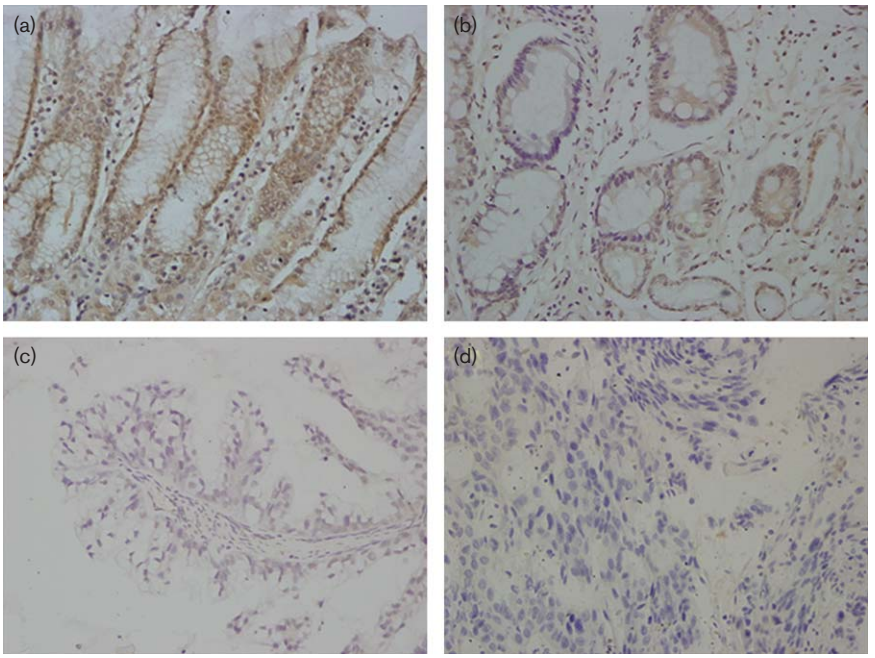
Prenyl diphosphate synthase, subunit 2 (*PDSS2*)-induced apoptosis in SGC790 cells. The apoptosis rate was measured by flow cytometric (FCM) assays at 48 h posttransfection. (a) Representation of typical FCM scattergrams of untransfected cells (001); cells transfected with pcDNA3.1 plasmid (2.5 μg per 4×10^5 cells) (002); cells transfected with pcDNA3.1-*PDSS2* plasmid (2.5 μg per 4×10^5 cells, 4.0 μg per 4×10^5 cells) (003 and 004). A two-parameter cytogram of log (FL1) (annexin) versus log (FL3) (propidium iodide, PI) was plotted. Early apoptotic cells (annexin V^+/PI^-) were detected in the lower right quadrant and late apoptotic cells (annexin V^+/PI^+) in the upper right quadrant. (b) Comparison of early and late apoptosis of cells at 48 h posttransfection. Data are represented as the mean ± SD of three experiments. $^*P < 0.05$ versus control. FITC, fluorescein isothiocyanate.

24 of 39) and only six of 39 (15.4%) remained high (++) in gastric tumor tissues. By contrast, *PDSS2* expression remains high (++) in the vast majority of normal gastric samples (91.7%, 11 of 12). Moreover, in poorly differentiated gastric cancer, the negative or low expression of *PDSS2* was 35% (seven of 20) or 55% (11 of 20) and in moderately differentiated gastric cancer, it was 10.5% (two of 19) or 68.4% (13 of 19), respectively. The higher the malignant degree of tumor, the lower the expression level of PDSS2 protein.

Discussion

Detailed mapping of LOH revealed at least two frequently deleted genomic regions on chromosome 6q16.3-6q23 and 6q26-27 which could harbor one or more TSGs [5,6]. The *PDSS2* gene is located on human chromosome 6q21 and encodes a predicted 399-amino acid protein, which Saiki *et al.* [9] identified as one subunit of decaprenyl diphosphate synthase. In the absence of PDSS1 or *PDSS2*, the enzyme is not functional and does not produce CoQ10 [10,11]. The gene product was hypothesized to be a tumor suppressor by Guan *et al.* in their study on the 6q deletion and rearrangement in a melanoma cell line [8]. In turn, studies on *PDSS2* gene expression profiling in normal and cancer tissue will provide essential information for the further investigation of this gene. Commonly, it is expected that TSGs will be transcriptionally suppressed or even inactivated by mutations, deletions, or loss of expression in tumors *in vivo*, whereas TSGs will be expressed highly in the corresponding normal tissue. Our immunohistochemical study showed that expression of *PDSS2* is downregulated in human gastric cancer and the expression level correlates with the stage of differentiation of the cancer. Eleven of 12 samples of normal gastric

Fig. 5



Immunohistochemical staining of the prenyl diphosphate synthase, subunit 2 (PDSS2) protein in tissue sections from normal gastric biopsies and cancer tissue biopsies. Note the cytoplasm staining of PDSS2 protein was stained brown by diaminobenzidine (magnification, × 400). Representative sections are shown in (a) normal gastric tissue with strong positive signal, (b) metaplasia with *PDSS2* expression, (c) moderately differentiated tumor with decreased *PDSS2* expression and (d) poorly differentiated tumor with absence of *PDSS2* expression.

tissue had high expression of levels of the PDSS2 protein. In contrast, 33 of 39 samples of gastric cancer tissues had negative or low expression levels of the PDSS2 protein. Clinically, a lower expression level of *PDSS2* corresponded to poor differentiation of gastric cancer (18 of 20). These data suggested a novel functions of *PDSS2* as a tumor suppressor or as a factor to regulate the progression of the malignant character of cancer cells.

To begin evaluating the possibility that PDSS2 might have anticancer activity, our immediate approach was to test if the gene has the ability of suppressing tumor cell growth *in vitro*. We constructed a eukaryotic expression plasmid containing full length *PDSS2* obtained from normal gastric tissue. Our results showed that *PDSS2* expression after transient transfection of expression construct showed a significant increase compared with endogenous PDSS2, which was expressed in untransfected cells and cells transfected with empty plasmid, especially the full length *PDSS2* expression (Fig. 1). This indicated construct *PDSS2* was expressed successfully in transfected SGC7901 cells. SGC7901 cell line we used in this study was derived from human moderately differentiated gastric cancer and expressed some amount of endogenous PDSS2. Commonly, it is necessary to use a cell line with no expression of PDSS2. According to AceView (*http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?*), however, the *PDSS2* gene is expressed in most of the normal tissues and some carcinomas at high level, 1.5 times the average genes. We selected several cell lines such as liver cell linesHepG_2 and 7721, Hela cell line, especially gastric cancer cell line MN45 and SGC7901, before we commenced our study. Unfortunately, we could not obtain a cell line with no expression of PDSS2, and the SGC7901 showed lower expression relatively. Therefore, we used the SGC7901 cell line in our first stage of study on this gene by assigning the control group. Comparing with the untransfected negative control or transfected with empty vector, our results revealed that high expression of exogenous PDSS2 resulted in suppression of cell proliferation in SGC7901 cells. After transfection of *PDSS2*, cell growth was markedly suppressed in a dose-dependent manner.

To further assess the effect of *PDSS2* on cell cycle progression, PI staining followed by fluorescence-activated cell sorter cell cycle distribution profiles was used to compare the distribution of cells in the G_1, S, or G_2/M stage of the cell cycle. The results showed that *PDSS2* could induce cell cycle arrest in the G_0/G_1 phase. The sub-G_1 population indicated apoptotic-associated chromatin degradation. Simultaneous staining with annexin V and PI can distinguish among intact cells, early apoptosis, late apoptosis, and cell death. The results of annexin V–PI flow cytometric detection in this study further confirmed that the SGC7901 cells with

transfected *PDSS2* were in the process of apoptosis. Meanwhile, there were also universal characteristics of apoptotic cells apparent in the *PDSS2* transfected cells, such as nuclear condensation and apoptotic bodies.

In summary, we addressed the possible role of PDSS2 protein in gastric tumorigenesis. We have provided evidence that *PDSS2* may be a potent gastric cancer growth suppressor *in vitro* acting through apoptosis pathways. The anticancer activity of *PDSS2* could be attributed in part to its inhibition of proliferation and apoptosis induction of cancer cells through causing cell cycle arrest in the G_0/G_1 phase. The antitumor effects reported here are valuable for further investigation. Many important components, however, remain undiscovered. The pathway(s) mediating gastric cancer cell growth suppression and apoptosis after the reexpression of *PDSS2* is unclear, and further studies will be necessary to elucidate the mechanism of suppressive effects of *PDSS2* in gastric cancer and other human tumors.

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