Interaction between p12^{CDK2AP1} and a novel unnamed protein product inhibits cell proliferation by regulating the cell cycle

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Abstract. Human p12^{CDK2AP1} protein is encoded by the cyclin-dependent kinase 2-associated protein 1 (CDK2AP1) gene. This protein suppresses cell growth, differentiation and angiogenesis in numerous types of carcinoma by interacting with certain cell cycle proteins, including CDK2 and DNA polymerase α/primase. p12^{CDK2AP1} exerts its functions predominantly through protein-protein interactions. Therefore, the identification of other p12^{CDK2AP1}-interacting proteins may clarify its role in cell cycle regulation and carcinogenesis. The aim of this study was to identify additional p12^{CDK2AP1}-interacting proteins. A novel unnamed protein product (UPP, BC006130) was identified through using a yeast two-hybrid system. The interaction of p12^{CDK2AP1} with the UPP was further verified by glutathione S-transferase pull-down and co-immunoprecipitation experiments *in vitro*. The qPCR results following overexpression and siRNA assays demonstrated that the expression levels of the UPP were mediated by the CDK2AP1 gene. Furthermore, overexpression of the UPP gene was shown to shorten the length of the G2/M phase of the cell cycle in normal and tumor cell lines in a flow cytometry assay. The results of human tumor xenografts experiments in Balb/c nude mice indicated that stable transfection with the UPP gene was able to inhibit tumor cell proliferation *in vivo*. Overall, this study identified and characterized a novel interactive protein of p12^{CDK2AP1}, which may inhibit cell proliferation by mediating the cell cycle. It expands the understanding of the mechanisms of p12^{CDK2AP1} and its potential as a cancer therapeutic target.

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Introduction

Cyclin-dependent kinase 2-associated protein 1 (CDK2AP1) is a putative growth suppressor gene originally identified and isolated from normal keratinocytes (1). The human CDK2AP1 gene is 1.6 kb long and is located on chromosome 12q24.31 (2). The human and rodent polypeptides encoded by the CDK2AP1 gene share 97% identity, while the mouse and hamster sequences are identical (3). Human CDK2AP1 encodes a 115-amino acid peptide with a molecular mass of 12.4 kDa (pI 9.62), namely p12^{CDK2AP1} (4). Previous studies have demonstrated that p12^{CDK2AP1} negatively regulates cell growth by sequestering the monomeric non-phosphorylated form of CDK2 and targeting it for proteolysis to reduce the active pool of CDK2 in cells (5). The protein also reduces CDK2-mediated retinoblastoma protein phosphorylation by reversing the activity of TGF-β (6). A previous study has demonstrated that miR-21 downregulates p12^{CDK2AP1} to stimulate cell proliferation and invasion (7). These observations indicate that p12^{CDK2AP1} acts as a growth suppressor through influencing mitosis, the S phase and the cell division cycle. A study has demonstrated that p12^{CDK2AP1} is also important in promoting Oct4 promoter methylation during murine embryonic stem cell differentiation and thereby downregulates Oct4 expression levels (8). In addition, p12^{CDK2AP1} has been shown to induce the epithelial-mesenchymal transition of hamster

cheek pouch carcinoma-1 cells by promoting the expression of Twist2 (9).

Other clinical studies have demonstrated that p12^{CDK2AP1} is associated with oral squamous cell carcinoma, breast cancer, esophageal squamous cell carcinoma, gastric cancer and colorectal cancer (10-14). It has also been identified that p12^{CDK2AP1} is involved in anti-angiogenesis modulation and the suppression of malignant biological interactions between prostate cancer and bone cells (15). The possibility of using p12^{CDK2AP1} as a therapeutic target has been suggested (16).

Proteins undertake numerous physiological functions, such as the regulation of enzymatic activities, the assembly of cellular components and signal transduction. The elucidation of protein-protein interactions (PPIs) and whole interaction networks is vital to understanding biophysical processes. It is well known that numerous types of cancer are caused by dysfunctions of certain protein interactions (17). Studies have demonstrated that a number of proteins interact with p12^{CDK2AP1} (18,19). However, due to the diverse properties of proteins and the very different characteristics of PPIs (20), the proteins that interact with p12^{CDK2AP1} are not yet fully understood.

In the present study, a yeast two-hybrid assay was used to screen the proteins possibly interacting with p12^{CDK2AP1} and a novel unnamed protein product (UPP) was identified. The interaction between p12^{CDK2AP1} and this UPP was verified by glutathione S-transferase (GST) pull-down and co-immunoprecipitation (Co-IP) assays. Subsequently, the functions of the UPP in the PPIs with p12^{CDK2AP1} were studied *in vitro* and *in vivo*. The results demonstrated that p12CDK2AP1 regulates the expression of the UPP and may be inhibit cell proliferation by interaction with it.

Materials and methods

Materials. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise mentioned. Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, Dulbecco's phosphate-buffered saline (PBS) and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA, USA).

Yeast strain and growth medium. The genotypes of the *Saccharomyces cerevisiae* strain MaV203 (Invitrogen) used in this study were as follows: MATα, leu2-3, 112, trp1-901, his3Δ200, ade2-101, gal4Δ, gal80ΔSPAL10::URA3, GAL1::lacZ, HIS3UAS GAL1::HIS3@LYS2, can1R and cyh2R. This strain contains a set of non-reverting autotrophic mutations. A blend of yeast extract, peptone, dextrose and adenine sulfate medium was used to grow the untransformed yeast strain. Synthetic dropout minimal medium (Clontech Laboratories, Inc., Victoria, Australia) with appropriate nutrients was used to separate the untransformed yeast strain from the transformed.

Plasmid construction. Human liver CDK2AP1 cDNA (Stratagene, La Jolla, CA, USA) was ligated between the *Eco*R I and *Xho* I sites of a pBD-GAL4 Cam phagemid vector (Stratagene), downstream of the GAL4 DNA-binding domain. A liver cDNA library was digested by an interference-resistant helper phage from the Cam Phagemid Vector kit (Stratagene) and transformed into the *Escherichia coli* (*E. coli)* XLOLR strain (Stratagene). The human CDK2AP1 cDNA and the UPP gene were cloned into the pGEX-4T-1 GST fusion expression vector (GE Healthcare, Piscataway, NJ, USA). As there was no commercially available antibody to detect the UPP, HA and FLAG protein tags were used to identify the expression of the UPP and p12^{CDK2AP1}. HA and FLAG mouse monoclonal antibodies (Zhongshan Golden-Bridge Biotechnology Co., Ltd., Beijing, China) were used to test the expression of HA-p12^{CDK2AP1} and FLAG-UPP in eukaryotes.

Yeast two-hybrid cDNA library screening assay. A human cDNA library was screened using the pBD-GAL4 plasmid, which was constructed with human full CDK2AP1 cDNA. The cDNA library bacterial XL1-Blue MRF was digested by a phage. The two vectors were then cotransfected into the MaV203 *S. cerevisiae* strain. Subsequently, positive clones were verified by distinguishing the His$^+$ phenotype from the Leu$^+$ and Try$^+$ phenotypes. The screening procedure fully complied with the manufacturer's instructions. Positive interaction was confirmed by testing the dropout culture media, and then the positive plasmid was transformed into *E. coli* JM109 for sequencing.

Pull-down assay. The pGEX 4T-p12^{CDK2AP1} and pGEX 4T-UPP plasmids were transformed into BL21 *E. coli* to express GST-p12^{CDK2AP1} and GST-UPP fusion proteins. Isopropyl β-D-1-thiogalactopyranoside (200 μl, 1 mM) was added to the flasks and the cells were induced for 5 h. The cells were then harvested with PBS. Pelleted cells were lysed by sonication in PBS and Complete Protease Inhibitor Cocktail (Roche, Mannheim, Germany). Following centrifugation at 12,000 x g for 5 min, the supernatants were applied to Glutathione Sepharose 4B (GE Healthcare). Thrombin Protease (GE Healthcare) was added to the GST-UPP tube for 30 min. The beads were pelleted, washed three times in PBS, resuspended in Laemmli sample buffer and analyzed by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Cell culture and transfection. Human 293T cells and HeLa cells were cultured under 37˚C, 5% CO_2 in DMEM supplemented with 10% FBS, penicillin (10,000 U/ml) and streptomycin (10,000 μg/ml). When the cells reached 50-60% confluence, Lipofectamine 2000 reagent (Life Technologies Corp., Rockville, MD, USA) was used to transfect the pcDNA 3.1-FLAG-UPP and pcDNA 3.1-HA-p12^{CDK2AP1} plasmids according to the manufacturer's instructions. The medium was changed after 5 h.

Co-IP and western blot analysis. A Protein G Immunoprecipitation kit was used to perform the Co-IP assay according to the instructions provided with the kit. The 293T cells were lysed in IP buffer 48 h after transfection. The lysate was divided into two spin columns and the samples were analyzed by SDS-PAGE. Proteins were transferred onto nitrocellulose membranes (Invitrogen) and then incubated with primary antibodies. The anti-HA antibody (1:10,000/2 μl) was applied to the UPP membrane, while the anti-FLAG antibody (1:10,000/2 μl) was applied to the p12^{CDK2AP1} membrane. After washing four times with TBST (20 mM Tris-HCl, pH 7.4; 137 mM NaCl and 0.1% (v/v) Tween 20), the membranes were incubated with horseradish peroxidase-conjugated goat

anti-mouse secondary antibodies. The ECL Western Blotting Detection system (Amersham Biosciences, Piscataway, NJ, USA) was used to detect horseradish peroxidase on the immunoblots. The dried membranes were exposed to Carestream Kodak Biomax MR film for 2 min at room temperature.

siRNA and qPCR assays. The siRNA sequences used were designed and purchased from Genepharma Co. (Shanghai, China), and were as follows: Forward, 5'-UCUUUUCCAAACUGAAUGCGC-3' and reverse, 5'-GCAUUCAGUUUGGAAAAGACA-3' for the UPP gene; and forward, 5'-AGGAGAUCAGACCCACGUATT-3' and reverse 5'-UACGUGGGUCUGAUCUCCUTT-3' for the $p12^{CDK2AP1}$ gene. The siRNA oligo was transfected into the 293T cell line with Lipofectamine 2000. After 24 h, the cells were harvested and divided into two centrifuge tubes. Total RNA from the cultured cells was isolated using TRIzol reagent (Life Technologies Corp., Carlsbad, CA, USA). The primers used to detect the expression levels were as follows: Forward, 5'-AACGGAACGGAATGC CAGATCCTA-3 and reverse, 5'-TTCAGAGCCAAGTGA ACCATGGGA-3' for $p12^{CDK2AP1}$; and forward, 5'-TGGTGC ACATCGTGTAACCTGTCT-3' and reverse, 5'-AAGCTT TCCTTCCTCAGCCACTCT-3' for the UPP. Following cDNA synthesis, qPCR was performed using the Bio-Rad Real-Time PCR Detection system (Bio-Rad, Hercules, CA, USA) with the human housekeeping cDNA GAPDH as the control. Radio immunoprecipitation assay buffer cocktail (Dingsheng Biotechnological Co., Beijing, China) was added to the tube containing 293T cells. Anti-mouse HA monoclonal antibody was used to perform western blotting to test the expression of $p12^{CDK2AP1}$. The human 293T, A-549, HeLa, MCF-7, ACC-M, fibroblast and human mesenchymal stem cell lines were incubated to examine the endogenous expression of the $p12^{CDK2AP1}$ and UPP genes. The comparative threshold cycle (CT) method was used to analyze relative changes in gene expression.

Flow cytometry and cell viability assay. The UPP-transfected 293T and HeLa cells were harvested at 12 and 24 h. Prior to flow cytometric examination, the cells were combined with propidium iodide (1 mg/ml) and RNase (50 μg/ml). The cell cycle was analyzed by flow cytometry (FACSCalibur; Becton-Dickinson, San Jose, CA, USA), and ModFit and CellQuest software was utilized. The plasmid pcDNA 3.1-FLAG-UPP was transfected into 293T cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (20 μl, 5 mg/ml) was applied to the wells after incubation for 24 h. Dimethyl sulfoxide (Amersco, Solon, OH, USA) was added to each well to dissolve the formazan crystals for 10 min. The samples were then transferred to a plate reader (ELx808; BioTek, Shanghai, China) and the absorbance at 570 nm was measured.

DNA methylation assay. The bisulfite sequencing PCR (BSP) method was used to examine the UPP gene promoter methylation in the 293T and HeLa cell lines. The methylation primers used to perform PCR were as follows: Forward, 5'-GTAGTG TGAGTGAGGGTTTTGATTT-3' and reverse, 5'-CTAACA ACAAATTACCCCAACTTTC-3'. Streptozotocin buffer (Tris-HCl 10 mM, pH 8.0; EDTA 4 mM; NaCl 0.4 M), SDS and protease K were applied to harvested 293T and HeLa cells. DNA extraction followed the standard procedure. $Na_2S_2O_5$ (5 mol/l) and hydroquinone mixture were used to prepare HeLa and 293T cell DNA. Subsequently, PCR was performed with the conditions as follows: 94˚C for 5 min, 94˚C for 40 sec, 56˚C for 40 sec and 72˚C for 50 sec for 34 cycles, and then 72˚C for 10 min. An E.Z.N.A. Cycle Pure kit (Omega Bio-Tek Inc., Norcross, GA, USA) was used to purify the PCR products. Subsequently, the PCR products were ligated into a pMD 18T vector (Takara Bio, Inc., Tokyo, Japan) for sequencing. BiQ Analyzer software was used to statistically analyze the results (21).

Human tumor xenografts in vivo. The animal experiment followed the Laboratory Animal Center Institutional Animal Care and Use committee guidelines of the Peking University Health Science Center. Nude mice (n=36; Balb/c nude, female, four weeks old) were divided into three groups at random. The HeLa cells which were stably transfected with the UPP gene by an Overexpression Lentivector (SBI, Mountain View, CA, USA) were screened by flow cytometry. HeLa-UPP cells were subcutaneously injected at a density of $1x10^5$ cells/ml into the armpits of the nude mice, with the HeLa cell line as the negative control and the blank plasmid-infected HeLa cells as the positive control. Once the tumors reached 3-5 mm, all the tumors were harvested. The weights and volumes of the tumors were then calculated at the same time.

Statistical analysis. All cell experiments were repeated three times. The data were calculated as the mean ± standard deviation and the statistical analyses were performed using SPSS Software, version 19.0 (SPSS, Inc., Chicago, IL, USA). The differences were considered to be statistically significant when $P<0.05$, as determined by Student's t-test.

Results

Identification of a novel interaction protein of $p12^{CDK2AP1}$ by a yeast two-hybrid screening assay. Interacting cellular partners of $p12^{CDK2AP1}$ were searched for by screening a human cDNA library using a bait construct with the full CDK2AP1 cDNA cloned in-frame through using a yeast hybrid system. Following screening, positive clones were identified by selecting with synthetic dropout minimal medium. A protein that activated yeast transcription was detected (Fig. 1A). The activating domain/library plasmids were confirmed by the cotransformation procedure, and the nucleotide sequence of the protein was obtained by cloning and sequencing. Through blasting the sequence in the NCBI BLAST database, a novel protein named UPP (BC006130) was identified. The UPP is encoded by the Loc93622 gene, which is located on 4q16.1, and includes 119 amino acids.

Verification of the interaction between $p12^{CDK2AP1}$ and the UPP in vitro. Considering the possibility of a false positive result in the yeast two-hybrid system, the interaction between $p12^{CDK2AP1}$ and the UPP was confirmed by GST-pull down. The prokaryotic expression plasmids pGEX-4T-$p12^{CDK2AP1}$ and pGEX-4T-UPP were constructed to express GST fusion proteins, and then the GST-$p12^{CDK2AP1}$ and GST-UPP proteins were purified by GST affinity chromatography (Fig. 1B and C). Subsequently,

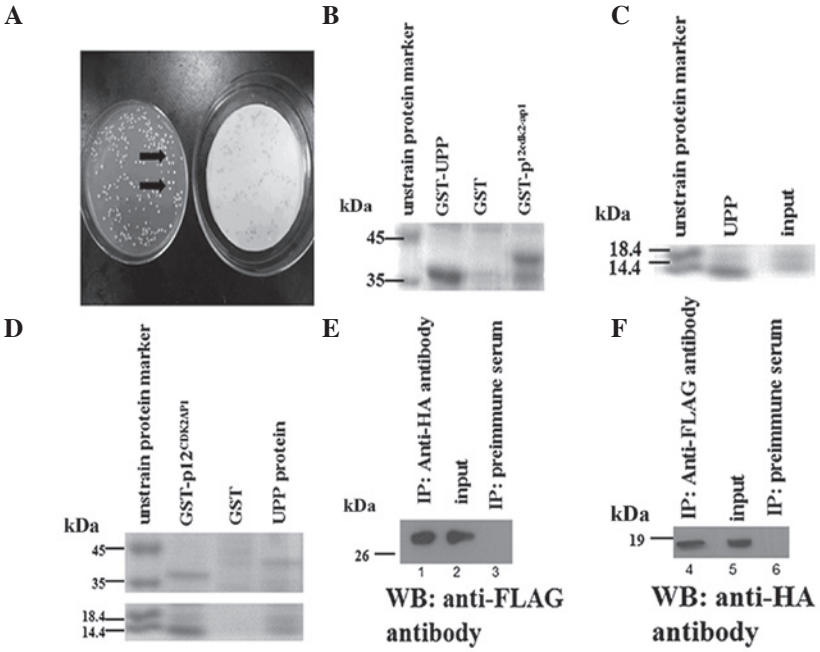

Figure 1. A novel protein interacting with p12^{CDK2AP1} was screened and confirmed. (A) The yeast-hybrid assay screened a protein interacting with p12^{CDK2AP1}. The positive clones are indicated by the arrows. (B) The GST-p12^{CDK2AP1} (39.4 kDa) and the GST-UPP (37 kDa) were purified by GST affinity column chromatography. (C) The purified UPP protein. (D) The pull-down assay demonstrated that GST-p12^{CDK2AP1} interacts with the UPP. (E and F) The Co-IP assay demonstrated that p12^{CDK2AP1} and the UPP co-immunoprecipitated in the cells. Preimmune serum was used as the control. UPP, unnamed protein product; CDK2AP1; cyclin-dependent kinase 2-associated protein 1; Co-IP, co-immunoprecipitation.

the GST tag of GST-UPP was cleaved by thrombin protease. The pull-down result supports the specific interaction of p12^{CDK2AP1} with the UPP (Fig. 1D). To confirm this interaction in mammalian cells, the interaction of p12^{CDK2AP1} and the UPP was examined using a Co-IP assay. The bound proteins were detected by western blotting using antibodies which were specific to HA and FLAG (1E and F). These results demonstrated that p12^{CDK2AP1} interacts with the UPP in cells.

Expression and methylation of the UPP gene. To analyze the endogenous expression of the UPP gene, non-neoplastic cell lines (293T, human mesenchymal stem cells and human fibroblast cells) and human cancer cell lines (ACC-M, A-549, MCF-7 and HeLa) were tested. The results of the qPCR demonstrated that the UPP and p12^{CDK2AP1} were similarly expressed (Fig. 2A and B), that is, the UPP expression levels were higher in the non-neoplastic cell types than in cancer cell lines. Due to the high GC ratio (63%) of the UPP gene, the UPP gene was searched in the NCBI database and a CpG island of the gene was identified. The methylation of the UPP gene was investigated in the 293T and HeLa cell lines. The DNA of 293T and HeLa cells was extracted and amplified by BSP methods. The sequencing results demonstrated that there were 31 methylation sites in the UPP gene. In the DNA of the HeLa cells, all the methylation sites were methylated, while the 293T DNA was not methylated (Fig. 2C).

CDK2AP1 mediates expression of the UPP gene. To investigate the association between CDK2AP1 and the UPP gene, siRNA and overexpression assays were performed. Following transfection of the p12^{CDK2AP1} siRNA oligo, the western blot analysis demonstrated the silencing effect of the CDK2AP1 gene (Fig. 2D). The results indicated that the UPP expression levels were decreased when the CDK2AP1 gene was interfered with (Fig. 2E), while the UPP siRNA did not downregulate the expression levels of the CDK2AP1 gene (data not shown). Subsequently, the association between CDK2AP1 and the UPP was evaluated by overexpressing the two genes in the 293T cell line. The results revealed that overexpression of the CDK2AP1 gene significantly increases the expression levels of the UPP gene (Fig. 2F), while overexpression of the UPP gene does not affect the expression of CDK2AP1 (data not shown). Thus, the UPP may function as a downstream effector of CDK2AP1.

Overexpression of p12^{CDK2AP1} and UPP modulates the cell cycle and inhibits cell vitality in vitro. p12^{CDK2AP1} mediates the cell cycle and affects cell vitality; thus, whether the UPP gene also shifts the cell cycle and is associated with cell vitality was investigated. The UPP and p12^{CDK2AP1} genes were transfected into 293T and HeLa cells. After the genes had been transfected for 24 h, the flow cytometry data indicated that the percentage of cells in the G2/M phase was decreased significantly from 11.13% to 6.16% in the 293T cells (Fig. 3A) and from 12.15% to 7.65% in the HeLa cells (Fig. 3B). The cell growth curve demonstrated that overexpression of the UPP also suppresses cell proliferation (Fig. 3C and D). After transfection for 5 days, the cell vitality results indicated that overexpression of the UPP decreased cell survival (Fig. 3E and F).

Stably-transfected UPP inhibits the growth of human tumor xenografts in vivo. To investigate the mechanisms of action of the UPP protein *in vivo*, a HeLa cell line stably transfected with the UPP gene (Hela-UPP) was constructed (Fig.4A and B) and transplanted in nude mice as xenografts. After 62 days, the

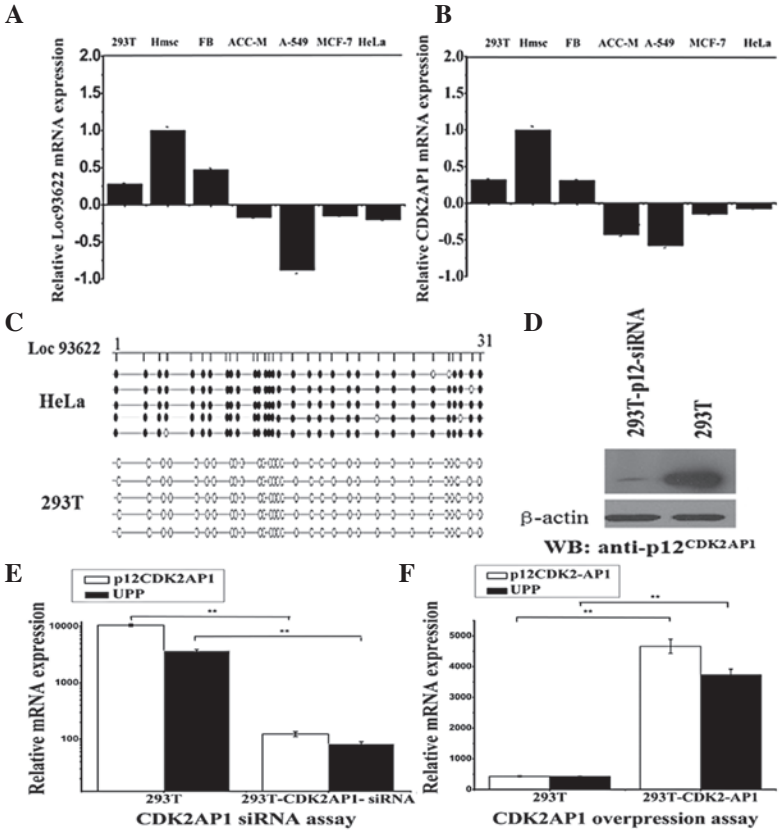

Figure 2. qPCR indicates the UPP and CDK2AP1 gene mRNA levels. (A and B) The UPP and p12^{CDK2AP1} gene expression levels, respectively, were detected in 293T, human mesenchymal stem (Hmsc), human fibroblast (FB), ACC-M, A-549, MCF-7 and HeLa cell lines. (C) The methylation of the Loc93622 gene in two cell lines, as indicated by the black dots. (D) Western blotting was used to test the effect of expression of p12^{CDK2AP1} siRNA. The UPP and p12^{CDK2AP1} mRNA expression levels in 293T cells (E) expressing p12^{CDK2AP1} siRNA and (F) overexpressing p12^{CDK2AP1}. **P<0.01. Human GAPDH was used as the control. CDK2AP1; cyclin-dependent kinase 2-associated protein 1; UPP, unnamed protein product.

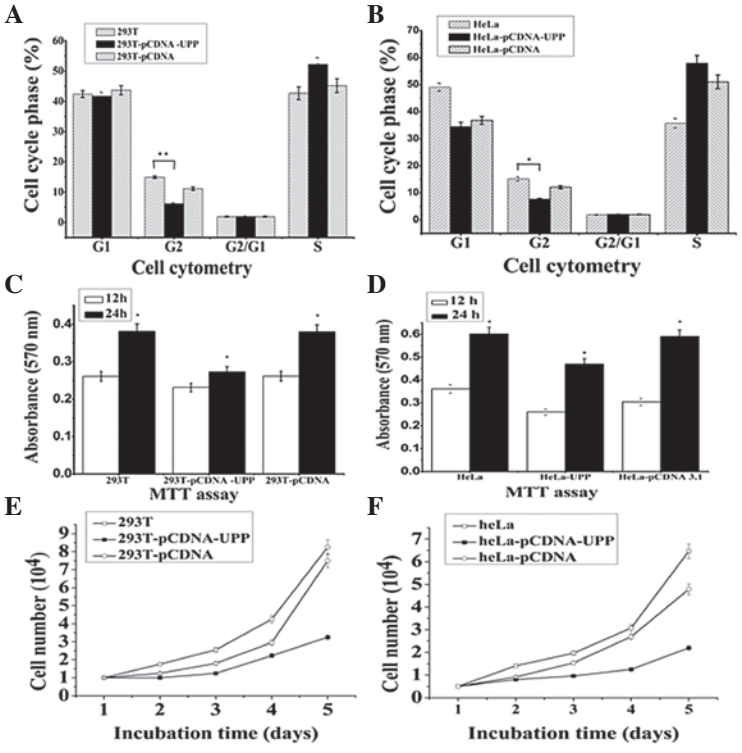

Figure 3. Overexpression of the UPP gene reduced the number of cells in the G2/S phase and affected cell vitality in 293T and HeLa cell lines. Cell cycle analysis of overexpression of the UPP gene in (A) the 293T cell line and (B) the HeLa cell line. *P<0.05. Vitality of the (C) 293T-UPP cells and (D) HeLa-UPP cells, with an empty vector as the control. Values are the mean ± SD of three independent experiments, *P<0.05. The number of (E) 293T-UPP cells and (F) HeLa-UPP cells after five days incubation. UPP, unnamed protein product.

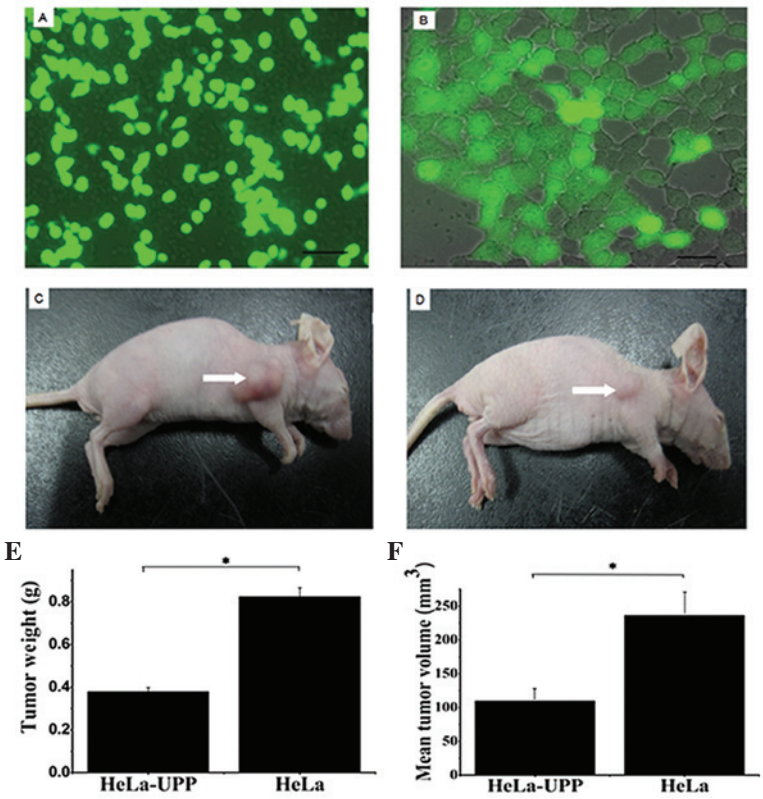

Figure 4. UPP gene suppresses cell proliferation in human tumor xenografts in nude mice. (A) The transfection of 293T cells with the UPP gene in virus packaging. (B) The stably-transfected UPP gene in HeLa cells following screening by flow cytometry. (C) HeLa and (D) HeLa-UPP cell tumor xenografts. The arrows mark the tumor xenografts. (E) The weight of the HeLa and HeLa-UPP cell tumor xenograft groups. *P<0.05. (F) The volume of the HeLa and HeLa-UPP cell tumor xenograft groups. *P<0.05. UPP, unnamed protein product.

xenograft tumors were harvested and fixed (Fig. 4C and D), and the weight and volume of the tumors were measured. The results demonstrated that the mean weight of the tumors of the HeLa-UPP cells (0.38±0.07 g) was lower than that of the control cells (0.77±0.11 g; Fig. 4E), and the mean volume of the tumors in the HeLa-UPP cells (109.62±20.40 mm^3) was also lower than that of the control group (228.62±76.58 mm^3; Fig. 4F). These results indicated that the UPP effectively inhibits the growth of human tumor xenografts.

Discussion

Mechanistic studies have demonstrated that p12^{CDK2AP1} binds to DNA polymerase α/primase protein, thereby inhibiting DNA replication (6). In addition, p12^{CDK2AP1} interacts with the monomeric non-phosphorylated form of the CDK2 protein to inhibit the S phase of the cell cycle (19). Thus, the PPIs of p12^{CDK2AP1} have a number of vital roles in physiological processes. However, the PPIs of p12^{CDK2AP1} require further study and the present study sought to identify proteins which interact with p12^{CDK2AP1}.

Through using the two-hybrid system assay, a novel protein (UPP, BC006130) was identified to interact with p12^{CDK2AP1}. The UPP is a 119-amino acid protein and its molecular mass is ~12.4 kDa. The protein is encoded by the Loc93622 gene, which is located on chromosome 4p16.1. Subsequently, the interaction was verified using pull-down and Co-IP assays. As the mechanism of the novel gene and protein has not been studied previously, the function of the protein was studied. To elucidate the association between the two genes, silencing and overexpressing assays were utilized. The results demonstrated that the expression of the UPP gene was mediated by the p12^{CDK2AP1} gene. However, the overexpression and silencing the UPP gene did not affect the expression of p12^{CDK2AP1}. This indicated that the p12^{CDK2AP1} gene may regulate the expression of the UPP and, as the p12^{CDK2AP1} gene is a potent transcription repressor, the UPP gene may be located downstream of the p12^{CDK2AP1} gene. Subsequently, the endogenous expression of the UPP gene in different types of cell was detected. The expression of the UPP gene was observed to be higher in non-neoplastic cell lines than in cancer cell lines and, similar to the expression pattern of the p12^{CDK2AP1} gene, the expression of the UPP gene also demonstrated tissue specificity. This result also suggested that p12^{CDK2AP1} may mediate the expression of the UPP gene.

Two studies have demonstrated that the CDK2AP1 gene is a core subunit of the methyl CpG binding domain protein 2 and 3 NuRD complexes (MBD2/NuRD and MBD3/NuRD) (22,23). The CDK2AP1 gene is putatively associated with a methylated promoter as a component of the MBD2/NuRD complex and is associated with carcinogenesis. Therefore, the CDK2AP1 gene may be involved in epigenetic mechanisms. In the present study, the BSP method was used to examine the methylation of the Loc93622 gene in the 293T and HeLa cell lines. The results demonstrated that the gene is hypermethylated in HeLa cells, which explained why the expression levels of the Loc93622 gene were downregulated in the HeLa cells. The result also verified that the Loc93622 gene was methylated in certain cell lines in the UCSC Genome Bioinformatics database (http://genome.ucsc.edu/). While the homologs of

the UPP, MORF4Ap1 and MORF4AP1L1 share sequence identity, they may have the similar function of interacting with histone acetylases and acetyltransferases to regulate chromatin dynamics (24). Further studies are required to address the association between the function of the CDK2AP1 gene and the Loc93622 gene methylation.

$p12^{CDK2AP1}$ inhibits cancer cell proliferation through decreasing the length of the S phase of the cell cycle and it also changes the proportions of cells in the G1 and S phases. During carcinogenesis, the transcriptional function of the $p12^{CDK2AP1}$-encoding gene (CDK2AP1) has been observed to be lost (25). Zolochevska *et al* (15) studied the CDK2AP1 mechanism in prostate cancer and they demonstrated that the gene inhibits cancer proliferation and changes the androgen-responsive pathway. In another study of the prostate cancer androgen receptor, it was identified that the UPP gene was upregulated in hormonal therapy-resistant prostate cancer (26). In the present study, overexpression of the UPP gene was performed to identify the functions of the UPP in the cell cycle. The results demonstrated that the length of the G2/M phase was decreased and that cell proliferation was suppressed. Moreover, the HeLa cells transfected with the UPP gene exhibited a lower tumorigenicity than the control in human tumor xenografts. As an interactive protein of $p12^{CDK2AP1}$, the UPP may participate in the inhibition of cancer cell proliferation with $p12^{CDK2AP1}$. This study speculated that $p12^{CDK2AP1}$ not only controls S phase but also modulates the G2/M phase of the cell cycle by interacting with the UPP.

In conclusion, the present study demonstrated that the UPP is a novel binding protein of $p12^{CDK2AP1}$. The interaction was verified by using *in vitro* and *in vivo* assays. Through studying the mechanisms of the interaction between the UPP and $p12^{CDK2AP1}$, it was demonstrated that the expression of the UPP gene was regulated by the $p12^{CDK2AP1}$ gene. Furthermore, $p12^{CDK2AP1}$ may be involved in the methylation of the UPP gene promoter. The novel UPP inhibits tumor cell proliferation by regulating the cell cycle. These findings extend the understanding of the anticancer mechanism of $p12^{CDK2AP1}$ in cancer therapy strategies.

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