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RNA Silencing Suppressor p19 Regulates The Expressions of Cell Cycle Related Genes*

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Abstract Besides its function as a pathogenicity determinant, the *Tombusvirus* P19 also serves as a suppressor of RNA interference (RNAi) by sequestering intracellular small RNAs such as the small interfering RNAs (siRNAs) and microRNAs (miRNAs). However, the effect of P19 on mammalian cells has not been evaluated before. A human embryonic kidney 293 cell line that stably expressed p19 (HEK293-p19) was generated. Flow cytometric analysis revealed that over-expression of P19 caused a significant accumulation of G2/M phase cells. Cell proliferation assays demonstrated a reduced DNA replication and cell growth in HEK293-p19 cells. Moreover, p19 altered the expression profiles of a number of cell cycle regulators in HEK293 cells, such as upregulation of cyclin A1, CDK2, CDK4, CDK6, p18, cyclin D2, p19INK4d and E2F1, and downregulation of p15, cyclin A2, cyclin B1 and cyclin E1. Thus, the data strongly indicate that p19 might influence multiple G2/M regulators to cause G2/M arrest.

Key words RNAi, siRNA, miRNA, p19, G2/M arrest, cell cycle, cyclin A1, cyclin A2
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The *Tombusviruses* genus is a single stranded positive RNA virus that belongs to *Tombusviridae* and contains at least 12 distinct species [1]. A remarkable feature of *Tombusviruses* is the ability of their 3′-proximal genomes to produce a subgenomic RNA (sgRNA) that encodes two overlapped viral proteins (p22 and p19). Moreover, the gene for p19 is completely embedded in the open reading frame of p22 [2]. The functions of these two proteins were elucidated in previous studies of the tomato bushy stunt virus (TBSV). P22 is an essential gene product required for cell-to-cell movement, while P19 is a major determinant of specific viral invasion and induction of symptoms in the host [3, 4]. One of the reasons that P19 has attracted so much attention recently is its unique role as a suppressor of small RNA-mediated gene silencing [5]. This mechanism for suppressing RNA-mediated gene silencing in the host organism is evolutionarily conserved not only in plant viruses, but also in animal and human viruses as it allows these pathogens to counteract the host's innate antiviral defense[6~9].

Although P19 is often regarded as the major determinant of pathogenicity in TBSV infection[4], the detailed mechanisms responsible for p19-mediated necrosis or apoptosis remain unclear. Necrosis or apoptosis as induced by the viral proteins has been studied in many animal viruses[10~12]. For instance, human immunodeficiency virus type-1 (HIV-1) encodes a well-conserved accessory protein called viral protein R (Vpr). Vpr can induce cell cycle arrest at the G2/M phase followed by cell apoptosis[13~15]. Vpr can also affect viral replication and this is positively correlated with the *in vivo* viral load[16, 17]. Thus, it is likely that one of the strategies used by viruses to target the host is by initially inducing G2/M arrest in the target cells.

A number of studies have demonstrated that G2/M cell cycle arrest could be caused by diverse mechanisms [18]. The phosphorylation state of cyclin B/CDK1 is frequently modulated by viral accessory proteins in direct or indirect ways. To induce G2/M arrest, Vpr may prevent cyclin B/CDK1 dephosphorylation and subsequent activation by directly activating the

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ATR (ATM- and Rad3-related protein) pathway[19]. In contrast, the human papillomavirus type (HPV) 16 E1^E4 protein can cause G2/M arrest by functioning as a direct CDK1 substrate, which may then prevent the activated cyclin B/CDK1 complex from entering the nucleus[20]. More recently, it has also been shown that HPV16 E1^E4 mediated G2/M arrest could be caused by the sequestration of the cyclin A/CDK2 complex in the cytoplasm[21].

In the current study, we showed that the RNA silencing repressor P19 from *Tombusviruse* could cause G2/M arrest and thus limit cell proliferation in human embryonic kidney 293 (HEK293) cells. After profiling the expression of cell cycle genes in HEK293 cells that stably expressed p19, we hypothesized that the P19-mediated G2/M arrest may be the result of altered gene expression of multiple cell cycle effectors during the G2/M checkpoint.

1 Materials and methods

1.1 Plasmids and primers

pCMV-Tag2B was purchased from Stratagene. The p19 gene was derived from pSG5mP19[22]. For the construction of the pCMV-Tag2B-p19 plasmid, the *Tombusvirus* p19 gene was first amplified from pSG5mP19 with a pair of primers (5′ ccgggatccttcgatatggaac 3′ and 5′ gccctgcagctagtgatttactc 3′). Then, the polymerase chain reaction (PCR) amplified products were subcloned into the *Bam*H Ⅰ and *Pst* Ⅰ sites of pCMV-Tag2B to form pCMV-Tag2B-p19. Human cyclin A2 and CDK2 were amplified from HEK293T cells using one-step RT-PCR kit (Takara). The pairs of primers were 5′ ttgaattctgatgttgggcaactctgcg 3′, 5′ ttggtaccgtacttggccacaacttctg 3′ for cyclin A2 and 5′ ttgaattctcatggagaacttccaaaag 3′, 5′ ttctcgagaggctatcagagtcgaagat 3′ for CDK2. The RT-PCR products were subcloned into *Eco*R Ⅰ/*Kpn* Ⅰ and *Eco*R Ⅰ/*Xho* Ⅰ sites of pCMV-Myc, respectively, to form the expression constructs of pCMV-Myc-cyclin A2 and pCMV-Myc-CDK2. The cloned genes were subsequently confirmed by DNA sequencing.

All primers used in the semiquantitation of reverse transcription-PCR (RT-PCR) reaction were synthesized from Sangon (Shanghai, China). The primers are listed as follows: cyclin B1, 5′ agtgaacaactgcaggccaa 3′and 5′ gtacatggtctcctgcaaca 3′; cyclin D1, 5′ atggaacaccagctcct 3′ and 5′ aggaagttgttggggct 3′; cyclin D2, 5′ atggagctgctgtgcca 3′ and 5′ tctttcggcccaactgg 3′; cyclin D3, 5′ atggagctgctgtgttg 3′ and 5′ agtccacttcagtgcca 3′; CDK4, 5′ tctcccttgatctgagaatg 3′ and 5′ agatacagccaacactccac 3′; CDK6, 5′ tcaggttgtttgatgtgtgc 3′ and 5′ tcagaagtaggtctttgcct 3′; p21, 5′ aaggtcagttccttgtgga 3′ and 5′ ttagggcttcctcttggaga 3′; p27, 5′ agatgtcaaacgtgcgagtg 3′ and 5′ gtgcttatacaggatgtcca 3′; CDK2, 5′ atccgagagatctctctgct 3′ and 5′ atccggaagagctggtcaat 3′; cyclin E1, 5′ tattgcaccatccagaggct 3′ and 5′ cagccaggacacaatagtca 3′; p15, 5′ tacggccaacggtggattat 3′ and 5′ gggtgggaaattgggtaaga 3′; p16, 5′ tgaaagaaccagagaggctc 3′ and 5′ gtgactcaagagaagccagt 3′; cellular p19INK4d, 5′ acatgctgctggaggaggtt 3′ and 5′ tctcttgctggagagggtga 3′; p57, 5′ aaccgctgggattacgactt 3′ and 5′ cttggcgaagaaatcggaga 3′; p18, 5′ atggatttggaaggactgcg 3′ and 5′ cttggtgttgagattggca 3′; cyclinA1, 5′ tctgaagcaatgcactgcag 3′ and 5′ catctgtgccaagactggat 3′; cyclinA2, 5′ atgagcatgtcaccgttcct 3′ and 5′ ctctcagcactgacatggaa 3′; CDC2, 5′ acactctggtacagatctcc 3′ and 5′ agaagacgaagtacagctga 3′; pRB, 5′ tgtcagagagagagcttggt 3′ and 5′ tttgctatccgtgcactcct 3′; p130, 5′ atgagcgaaagctacacgct 3′ and 5′cacagcagcaggtgataaga 3′; p107, 5′ aactacagcctagagggaga 3′ and 5′ gcagttcacacagtacagca 3′; E2F-1, 5′ tcgcagatcgtcatcatctc 3′ and 5′ attcatcaggtggtccagct 3′; β-actin: 5′ cacactgtgcccatctacga 3′ and 5′ ctgcttgctgatccacatct 3′.

1.2 Cell culture

Human embryonic kidney 293 (HEK 293) cells were cultured in Dulbecco′s Modified Eagle Medium (GBICO) supplemented with 10% fetal calf serum and were maintained in a 37℃ incubator containing 5% CO_2.

1.3 Antibodies

Anti-β-actin, anti-Myc and anti-Flag antibodies were purchased from Santa Cruz Biotechnology. Anti-cyclin A, anti-cyclin E1, anti-CDK1/cdc2, anti-CDK2, and anti-p15 antibodies were acquired from Bioworld (Beijing, China). Goat anti-mouse or anti-rabbit Ig secondary antibodies labeled with horseradish peroxidase were purchased from Zhongshan (Beijing, China). Rhodamine (TRITC)-conjugated goat anti-rabbit IgG antibody was purchased from Zhongshan.

1.4 Transfection and generation of stable cell lines

HEK293 cells were plated onto 35 mm^2 dishes at a density of 3×10^6 cells/ml for 48 h before transfection. After the cells were 80% confluent, we delivered approximately 4 μg of DNA into the cells using the VigoFect reagent (Vigorous Biotechnology).

Transfected cells were selected after being cultured in the presence of 1 200 mg/L G418 for 21 days. The selected resistant clones were then maintained in complete medium plus 300 mg/L of G418.

1.5 Thymidine incorporation assay

About 5×10^4 stable cells (293-vec and 293-p19) were plated onto each well in a 96-well plate. On each test day, 3.7×10^{10} μBq of [^{3}H] thymidine was added to each well 5 h before harvesting. Six repeats were performed for each sample at each time point. After 5 h of incubation, the cells were collected and washed with 1× PBS. They were then resuspended in 150 μl of 5% trichloroacetic acid. The cell pellets were then lysed in 75 μl of 0.5 mol/L NaOH/0.5% SDS and the incorporated radioactivity was counted with a scintillation counter.

1.6 Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from the cultured cells using the TRIzol reagent (Invitrogen). 1 μg sample of RNA was used as the template. RT-PCR was conducted using a one-step RT-PCR kit (Takara Biotechnology) according to the manufacturer's instructions. The reverse transcription was conducted at 50℃ for 40 min. After denaturing the DNA at 94℃ for 2 min, the reaction products were amplified by PCR at a condition of 94℃ for 30 s, 50℃ for 30 s, and 72℃ for 1 min, for 20 or 30 cycles.

1.7 Western blot assay

The cells were lysed in lysis buffer containing 1% NP-40, 50 mmol/L Tris-HCl (pH 7.5), 120 mmol/L NaCl, 200 μmol/L $NaVO_4$, 1 mg/L leupeptin, 1 mg/L aprotinin, and 1 μmol/L PMSF. After separation, the proteins were transferred to a membrane, which was first probed with a primary antibody, and then incubated with a horseradish peroxidase-conjugated secondary antibody. The blots were then visualized using an ECL kit (Santa Cruz Biotechnology).

1.8 Immunostaining

The 293 stable cells were first plated onto slides in 35 mm dishes one day before immunostaining. On the test day, the cells were fixed in 4% paraformaldehyde. After permeabilizing the cell membranes with 0.5% Triton X-100, the fixed cells were blocked with 10% fetal calf serum (FBS) before incubation with an anti-Flag antibody (1∶100 dilution). After extensive washing with 0.1% Tween 20 in 1× phosphate buffered saline (PBS), the cells were probed with a Rhodamine (TRITC)-conjugated goat anti-rabbit IgG antibody and visualized with a laser-scanning confocal microscope (Olympus Optical Co. Ltd).

1.9 Flow cytometric analysis on cell cycle distribution

Cells were collected from transfected culture plates and fixed in pre-cooled 70% ethanol at 4℃ for 1 h. Subsequently, the cells were digested with RNaseA at 37℃ for 20 min. Finally, 100 μl of propidium iodide (PI) solution (50 mg/L) was added to the cell suspension and cells were stained at room temperature for 10 min. The reaction products were measured using the FACSARIA flow cytometer (Becton Dickenson).

2 Results

2.1 Expression analysis of p19 in HEK293 stable cells

To facilitate the study of microRNAs, we constructed a HEK293 cell line that stably expressed the p19 gene (HEK293-p19). A stable cell line transfected with the vector alone (HEK293-vec) served as the control. p19 gene expression was confirmed using both reverse transcription polymerase chain reaction (RT-PCR, Figure 1a) and Western blot analysis (Figure 1b). Immunostaining was used to detect the intracellular localization of P19. Figure 1c shows P19 protein to be exclusively expressed in the cytoplasm.

2.2 P19 limits proliferation and causes G2/M arrest in HEK293 cells

Surprisingly, we found that the growth rate of HEK293-p19 cells was significantly slower than that of control HEK293-vec cells. To quantify the differences in growth, we plated equal amount of the stably expressing cells (~5×10^4 cells) onto each well in a 96-well plate. Cells were collected and counted 1, 2, 3 and 4 days post-inoculation. The number of HEK293-p19 cells was significantly lower than the number of HEK293-vec control cells after 2 days of incubation. After 3 days of incubation, the number of HEK293-p19 was only the half number of control cells (Figure 1d). The [^{3}H] thymidine incorporation assay demonstrated a dramatic decrease in DNA replication ((859 ± 96) cpm versus (1 456 ± 175) cpm) in HEK293-p19 cells on the fourth day of incubation compared to controls (Figure 1e). We then performed flow cytometry to detect whether cell cycle distribution was altered by the expression of the p19 gene.

Figure 2a shows an example of the experimental results that we obtained from the cell cycle analysis. After quantification, Figure 2b showed that the proportion of HEK293-p19 cells in the G2/M phase was significantly higher than that of the control cells ((19.00 ± 0.36)% versus (14.10 ± 0.46)%, P=0),

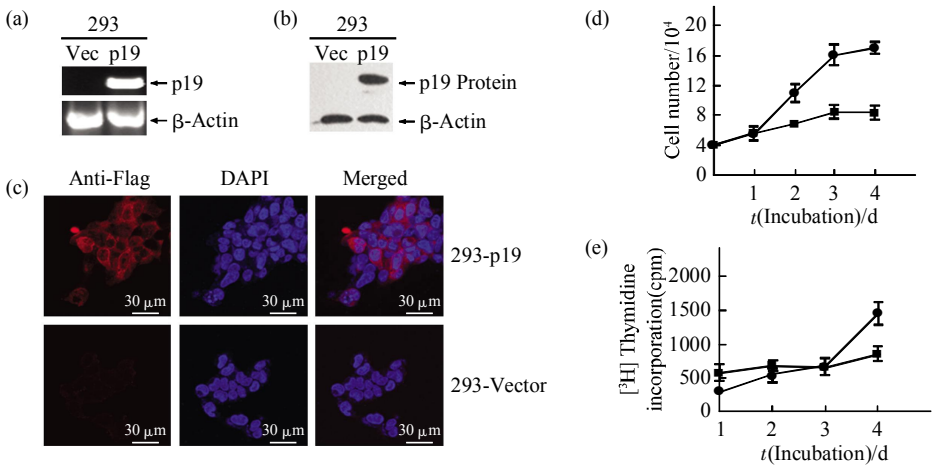

Fig. 1　Characterization of the p19-expressing cell line

HEK293 cells were stably transfected with plasmids pCMV-Tag-2B and pCMV-Tag-2B-p19 under the selection of G418 to generate neomycin resistant cell lines 293-vec and 293-p19, respectively. RT-PCR (a), Western blot (b) and immunostaining (c) were performed to detect p19 expression in 293 stable cells. (d) Measurement of cell growth. Cell growth potential was determined by calculating the absolute cell number every 24 h after initial plating in a 96-well plate with a fixed cell concentration. Each value represents the $\bar{x} \pm s$ of six repeated trials. (e) [^{3}H] thymidine incorporation assay. Samples were collected at 1, 2, 3 and 4 days after the initial plating in a 96-well plate with a fixed cell concentration. Each value represents the $\bar{x} \pm s$ from six repeats. ●—●: 293-Vec; ■—■: 293-p19.

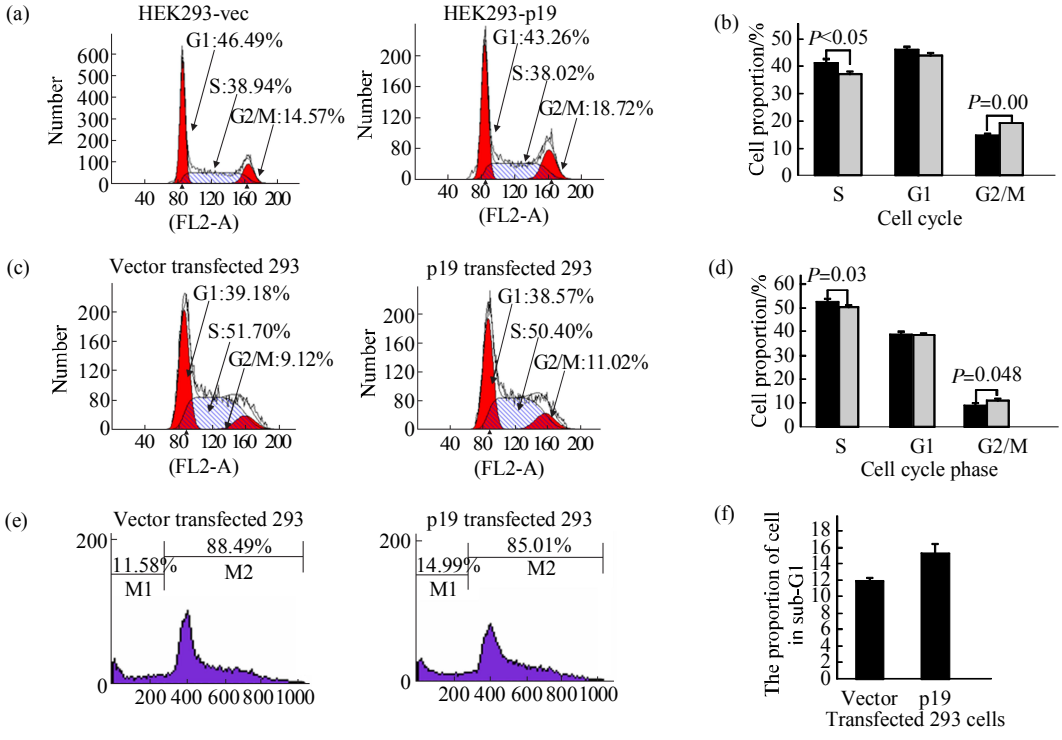

Fig. 2　The negative effects of p19 on cell cycle progression and cell survival

(a) The stable 293-vec and 293-p19 cells were collected and analyzed with flow cytometry after staining with propidium iodide. Arrows indicate the percentage of cells in a special cell cycle phase. (b) Quantification of cell cycle stages in (a). Each value is represented as $\bar{x} \pm s$ of triplicated tests. Similar results were obtained in two independent experiments. ■: 293-Vec; □: 293-p19. (c) The human embryonic kidney 293 cells were transiently transfected with the pCMV-Tag2B-p19 or pCMV-Tag2B (vector alone). After 48 h transfection, the cells were fixed, stained with PI and subjected to flow cytometric analysis. (d) Quantification of cell cycle distributions in (c). Values are represented as $\bar{x} \pm s$ of triplicated tests from one experiment. Similar results were obtained in at least two independent experiments. ■: Vector; □: p19. (e) The vector and p19 transfected 293 cells were also subjected to apoptosis analysis as roughly estimated by the proportion of sub-G1 cells (gate M1). (f) Quantification of sub-G1 cell populations assayed in (e). Values are represented as $\bar{x} \pm s$ of triplicated tests from one experiment. Similar results were obtained in at least two independent experiments.

whereas the proportion of HEK293-p19 cells in the S phase was significantly lower than that of the control cells ((37.00 ± 0.86)% versus (40.00 ± 1.82)%, $P < 0.05$). To further confirm the result, 293 cells were transiently transfected with equal amounts of plasmids pCMV- Tag2b and pCMV-Tag2b-p19. After 48 h transfection, the cells were fixed, stained and subjected to flow cytometric analysis. In consistent with the results of stable cells, delivering p19 increased G2/M population (Figure 2c). After quantitation, the result demonstrated a significant increase in G2/M phase cells and a marked decrease in S phase cells (Figure 2d). Since cell cycle arrest is often associated with apoptosis. We also measured the proportion of sub-G1 population in these p19 transfected 293 cells. Indeed, Figure 2e, 2f demonstrated a marked increase in sub-G1 cells after transfecting p19 into 293 cells, indicating that overexpression of p19 induces apoptosis. Overall, the results showed that p19 was able to induce G2/M arrest and apoptotosis in HEK293 cells.

2.3 RT-PCR analysis on mRNA expression profile of the major cell cycle regulators in HEK293-p19 cells

We next addressed which cell cycle-related genes might be affected as a result of p19 over-expression. To this end, we selected and systematically analyzed the expression levels of 22 cell cycle-related genes (p15, p16, p18, p19INK4d, p21, p27, p57, p107, p130, cyclin B1, cyclin A1, cyclin A2, cyclin B1, cyclin E1, cyclin D1, cyclin D2, cyclin D3, CDK2, CDK4, CDK6, CDC2, E2F-1 and RB) using semiquantitation RT-PCR. To avoid overlooking positive results, we conducted PCR for 20 and 35 cycles. Among the 22 different genes analyzed, the mRNA expression of twelve cell cycle related genes (cyclin B1, cyclin E1, cyclin A1, cyclin A2, CDK2, p18, CDK4, CDK6, cyclin D2, p19INK4d, E2F-1 and CDC2) was altered by p19 expression (Figue 3a). The relative density of each band was determined by AlphaEaseFC program

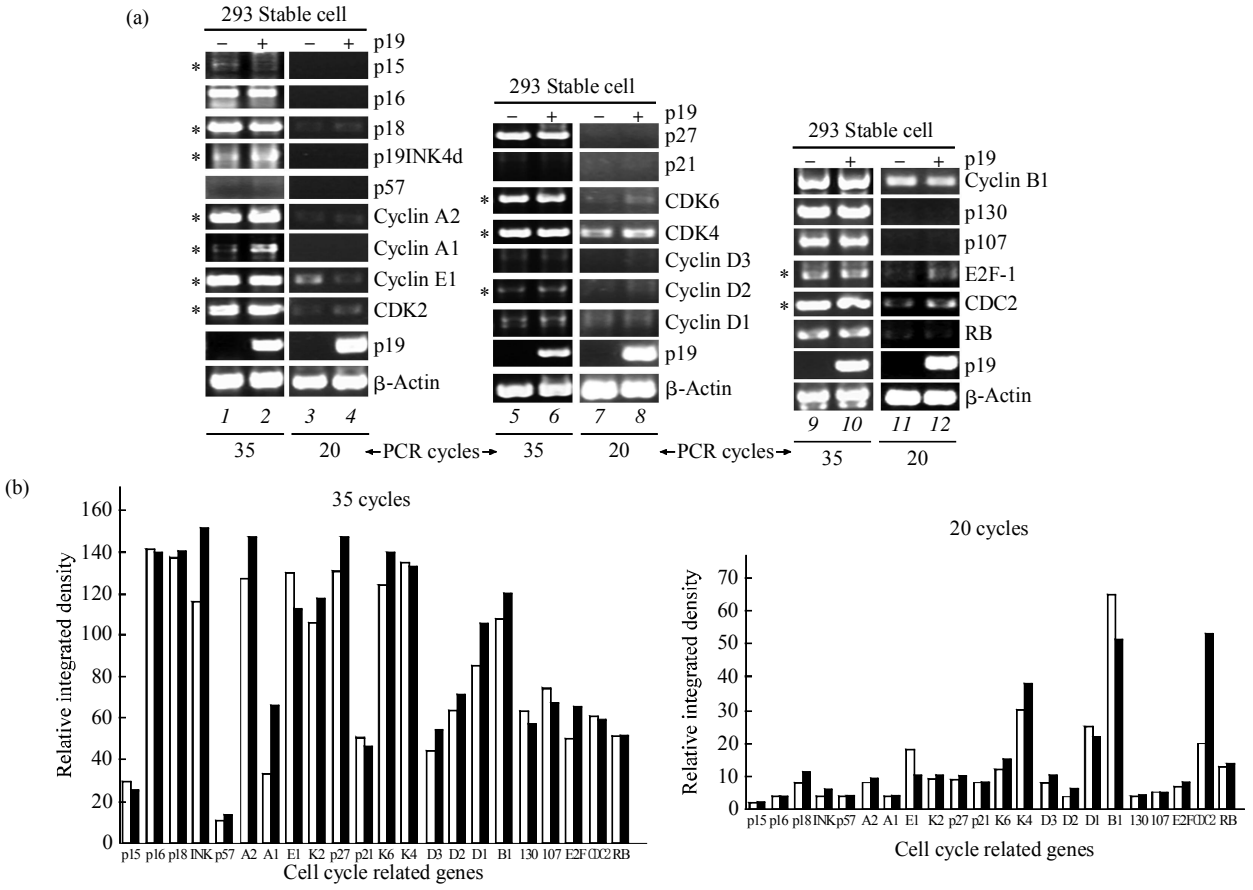

Fig. 3 The gene expression profiles of 293 stable cells were determined by RT-PCR analyses

(a) Total RNAs were extracted from 293-vec and 293-p19 stable cell lines. A one step RT-PCR was conducted for 20 or 35 cycles to amplify 24 different genes (**Materials and methods**). The reaction products were separated on a 1% agarose gel and subsequently photographed. Stars marked the significantly different genes between 293-vec and 293-p19 cells. (b) Quantification of the expression levels of the RT-PCR results presented in (a). Relative integrated density was determined by AlphaEaseFC system with automatic background subtraction. The results of RT-PCR for both 35 and 20 cycles are shown. INK, A2, A1, E1, K2, K6, K4, D3, D2, D1, 130, 107 stand for p19 INK4d, cyclin A2, cyclin A1, cyclin E1, CDK2, CDK6, CDK4, cyclin D3, cyclin D2, cyclin D1, p130 and p107, respectively. □: Vec; ■: p19.

with automatic background subtraction. The results in Figure 3b showed that p19 downregulated p15, cyclin B1 and cyclin E1 expressions and upregulated cyclin A1, cyclin A2, CDK2, p18, CDK4, CDK6, cyclin D2, p19INK4d, E2F-1 and CDC2 expressions (Figure 3b).

2.4 P19 influences the protein expressions of cell cycle regulators that potentially contribute to G2/M arrest

To further confirm the RT-PCR results, we used Western blot analysis to test the effects of p19 on cell cycle related proteins. Two sources of cell lysates were prepared: one from stably transfected HEK293 cell lines (293-vec and 293-p19) and the other from 293T cells transiently transfected with pCMV-tag2B and pCMV-tag2B-p19 (Figure 4a). Consistent with the RT-PCR results, there was a dramatic downregulation of cyclin E1 protein expression and a milder decrease in p15 expression in both the 293-p19 stable cell lines and p-19 transfected cells (Figure 4a, 4b). CDK2 was significantly up-regulated in both the 293-p19 stable cell lines and p-19 transfected cells (Figure 4a, 4b). In contrast, there was only a mild increase in CDC2/CDK1 expression in the transfected cells, whereas no induction of CDC2/CDK1 protein expression was detected in the 293-p19 stable cell lines (Figure 4a, 4b). There are two forms of human cyclin A proteins: cyclin A1 and A2. The initially identified cyclin A was later named cyclin A2[23]. It was notable that the mRNA level for cyclin A2 (cyclin A) was slightly elevated (Figure 3a, 3b), but the protein levels were markedly decreased after the expression of P19 in both 293 stable cell lines and transfected 293T cells (Figure 4a, 4b). To further validate the results, both cyclin A2 and CDK2 were cloned from 293T cells. After co-transfecting p19 with Myc-cyclin A2 and Myc-CDK2 into 293 cells, the cell lysates were probed with anti-Myc antibodies. Figure 4c demonstrated that p19 could decrease the steady state levels of Myc-cyclin A2 proteins in a dose-dependent manner, while increased CDK2 expression. Overall, the results clearly demonstrated that p19 was able to regulate a number of cell cycle related genes that might be important for the G2/M phase.

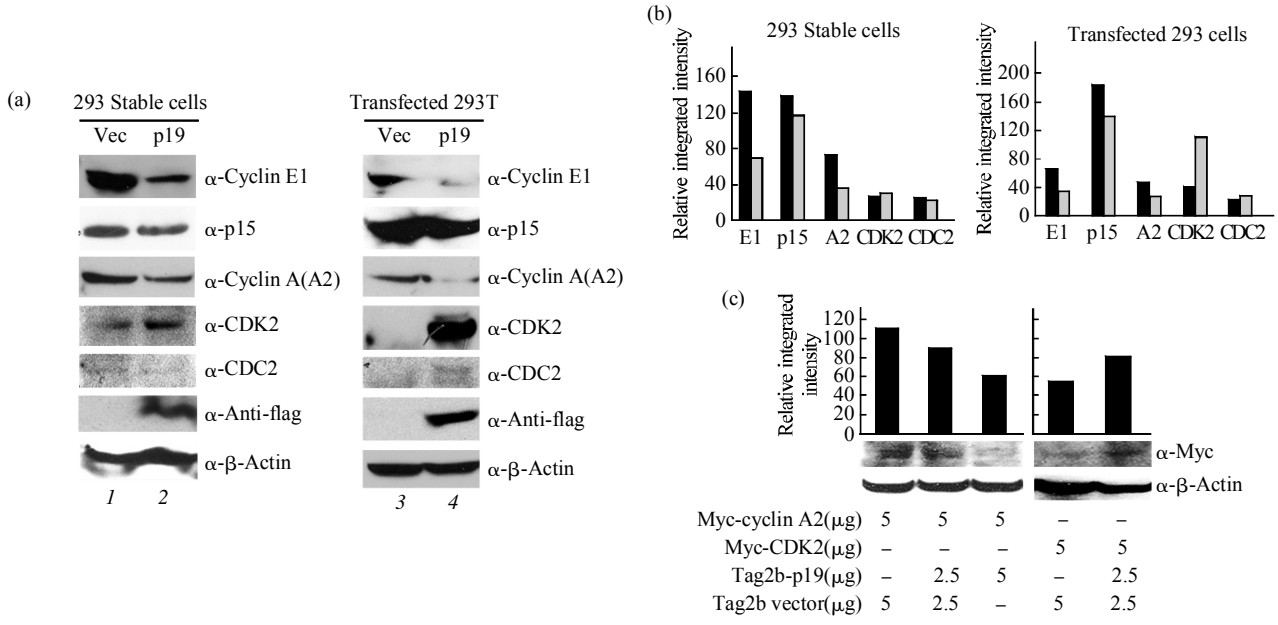

Fig. 4 Western blot analysis on p19 regulated cell cycle gene products

(a) Cell lysates were prepared from either 293 stable cell lines or transiently transfected 293T cells. Primary antibodies against cyclin E1, p15, cyclin A, CDK1 (CDC2), CDK2, Flag or β-actin were used to detect targeted gene expressions. The protein levels of β-actin served as loading controls. (b) Quantification of the protein levels of cell cycles related genes using AlphaEaseFC system with automatic background subtraction. E1 and A2 stand for cyclin E1 and cyclin A2, respectively. ■: Vec; □: p19. (c) The effects of p19 on cyclin A2 and CDK2 expression. About 5 μg of either Myc-cyclin A2 or Myc-CDK2 was co-transfected with different doses of p19 plasmid DNAs into 293 cells. Equal amount of protein lysate was separated and blotted with anti-Myc antibody, while β-actin expression was assayed as a loading control.

3 Discussion

P19 is a well-known RNA silencing repressor. It was isolated from a plant virus belonging to the *Tombusviruses* genus and has been widely used as a blocking agent to reverse small RNA mediated gene repression. However, the potential impact of p19 on mammalian cell functions has not yet been evaluated. We initially constructed a p19 stable cell line in human embryonic kidney 293 cells (293-p19) and attempted to test its effect on small RNA mediated gene silencing. To our surprise, 293 cells stably expressed p19 significantly attenuated the rate of cell growth and proliferation when compared to the vector control cells (293-vec). Further analysis revealed that the growth delay was associated with cell cycle arrest at the G2/M phase. Gene expression profiling on the cell cycle related genes indicated that p19 might have caused cell cycle arrest by directly or indirectly targeting multiple cell cycle regulators during the G2/M transition.

Compared with the high levels of cyclin A (A2) expression in human tissues and cell lines, cyclin A1 exhibits a more restricted expression pattern, being predominantly expressed in the testis, hematopoietic progenitor cells and myeloid leukemia cell lines[24, 25]. Cyclin A1 is also essential for meiosis in male spermatogenesis[26]. Using a more sensitive RT-PCR approach, it has been shown that even though as a prevalently expressed gene, cyclin A1 protein product is expressed extremely low in many tissues and tumor cell lines[24]. Cyclin A1 is complexed with CDK2, but is not associated with CDC2/CDK1, CDK4 or CDK5[24]. A p53-mediated up-regulation of cyclin A1 could induce G2/M arrest and apoptosis in a number of tumor cell lines[27]. Consistent with these observations, we have demonstrated here that the forced expression of p19 upregulated both the cyclin A1 and CDK2 gene products during p19-mediated G2/M arrest, indicating that a dysfunction in the cyclinA1/CDK2 complex may partially account for this cell cycle block.

Viral accessory proteins usually cause G2/M arrest by targeting multiple cell cycle-related genes[18]. The mechanisms responsible for the G2/M arrest involve various disruptions on the proper functions of the cyclin B/CDK1 complex. Whether p19 can directly interact with the cyclin B/CDK1 complex is presently unknown. Over-expression of p19 markedly increased CDC2/CDK1 mRNAs, however only slightly enhanced its protein level (likely the phosphorylated form of CDK1. The upper band, Figure 4 in lane *4*). In contrast, p19 potentially downregulated cyclin B1 mRNA expression (Figure 3). Thus, p19 may directly block normal dephosphorylation of CDC2/CDK1 during the late G2 phase. Alternatively, p19 might directly destabilize CDC2/CDK1 protein stability and subsequently prevent the functions of cyclin B/CDK1 complex. In addition, the cyclin A/CDK2 and cyclin A/CDK1 complexes are required for progression into the S and G2/M phases, respectively[28]. During a p53-mediated G2/M arrest, a decreased level of cyclin A (A2) was observed in head and neck carcinoma cells[29]. Interestingly, we found that the addition of p19 did not change the level of cyclin A2 mRNA, but rather caused a dramatic decrease in its protein level (Figure 3, 4). This indicated that a post-translational modification of Cyclin A2 protein might have occurred as a result of p19 overexpression. Therefore, a dramatic down-regulation of Cyclin A2 may contribute to p19 mediated the G2/M arrest.

Overall, the present study has demonstrated that the widely used gene silencing repressor p19 could cause G2/M arrest in HEK293 cells. This effect may be due to the alterations of a number of cell cycle regulators that are important for the G2/M transition.

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RNA 沉默抑制子 p19 调控细胞周期相关蛋白表达 *

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摘要 番茄丛矮病毒的 P19 蛋白不仅是一个重要的病毒致病因子，而且还可作为 RNA 干扰(RNAi)的抑制子．这种作用是通过限制细胞内的小 RNA，比如小干扰 RNA(siRNAs)和微 RNA(miRNAs)来实现．但是目前对 P19 蛋白在哺乳动物细胞上的作用还未见报道．构建了一株 p19 稳定表达的 293 细胞系，即 293-p19．流式细胞仪分析发现在 293 细胞中过量表达 P19 蛋白可显著引发细胞周期的 G2/M 阻滞．细胞增殖实验显示，293-p19 细胞的 DNA 复制及细胞生长均受到显著的抑制． 此外，研究还发现 p19 可使人胚肾 293 细胞内的细胞周期调控子的表达谱发生改变． 其中包括上调 cyclin A1，CDK2，CDK4，CDK6，p18，cyclin D2，p19INK4d 和 E2F1，及下调 p15，cyclin A，cyclin B1 和 cyclin E1 的表达．上述研究结果提示，p19 有可能靶向多个 G2/M 调控蛋白从而引发细胞的 G2/M 阻滞．

关键词 RNAi，siRNA，miRNA，p19，G2/M 阻抑，细胞周期，cyclin A1，cyclin A2
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