

Original Research

## Construction of recombinant adenovirus Ad-rat PLC $\gamma$ 2 and its effects on apoptosis of rat liver cell BRL-3A in vitro

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**Abstract:** Although the role of PLC $\gamma$ 2 in apoptotic response has been reported, too little is known about whether PLC $\gamma$ 2 induces liver cell apoptosis during liver regeneration. Therefore, this study firstly packaged Ad-PLC $\gamma$ 2 recombinant adenovirus and primarily evaluated its effect on apoptosis of rat liver cell BRL-3A in vitro. Following ten days of co-transfection of pHBAAd-MCMV-GFP-PLC $\gamma$ 2 and pHBAAd-BHG into HEK293 cells, viral cytopathic effect (CPE) was apparent. Following three rounds of amplification, tissue culture infectious dose 50 (TCID<sub>50</sub>) assay showed that the titer value reached  $1 \times 10^{10}$  PFU/mL. After 24 h of transfection of recombinant adenovirus into BRL-3A cells, transfection efficiency of adenovirus into BRL-3A cells was above 90% when observed under fluorescent microscopy. qRT-PCR and Western blot assays showed mRNA and protein levels of PLC $\gamma$ 2 were significantly elevated in the transfected BRL-3A cells. Flow cytometric analysis showed that, compared with the control and Ad-GFP groups, cell apoptosis rate of Ad-PLC $\gamma$ 2 group were significantly increased ( $P < 0.01$ ), and the cell cycle in Ad-PLC $\gamma$ 2 group was arrested at G1 phase which was manifested by a marked increase ( $P < 0.01$ ) in the percentage of G1 phase cells and a great decrease ( $P < 0.01$ ) in the percentage of S and G2/M phase cells. It was concluded from above results that recombinant adenovirus Ad-PLC $\gamma$ 2 was packaged successfully, and could promote cell apoptosis by arresting the transition from G1 to S phase of BRL-3A cells.

**Key words:** Rat phospholipase C gamma 2, Recombinant adenovirus construction, Gene transfection, Cell apoptosis, Cell cycle.

### Introduction

After partially resection, the recovery of liver mass is regulated by the functional needs of the organism (1,2). When hepatocyte number came back to a degree sufficient to assure normal hepatic contribution, liver regeneration would be terminated (3). For the successful completion of liver regeneration, the contribution of terminal phase is fully as important as that of initial phase. In recent thirty years, most researches have been focusing on the mechanism controlling the initiation of liver regeneration (4); whereas the mechanism controlling the terminating phase has not been investigated to the same extent as initial phase (5). Evidences have pointed out that more hepatocytes mass than needed were generated at the end of regeneration, and a certain degree of apoptosis was needed to precisely adjust liver mass (6). Some candidate pro-apoptotic signals have been identified as potential contributors to regeneration termination. For instance, TGF- $\beta$ 1 has been found to hamper hepatocyte proliferation by inhibiting DNA replication (7); Activin A can control hepatocyte mass via promoting hepatocyte apoptosis (8); NGF plays a significant role in positive regulation of apoptosis in liver stellate cell (9). More and more factors related to termination are discovered as the investigation proceeds, among which phospholipase C gamma 2 (PLC $\gamma$ 2) might be implicated in cell apoptosis. It has been shown that PLC $\gamma$ 2 level was elevated during gastric cancer cell apoptosis induced by TPA (10), suggesting its positive role in tumor cell death. Also, Tomlinson *et al.* reported the absolute necessity of PLC $\gamma$ 2 for BTK:ER-induced apoptosis responses. However, whether PLC $\gamma$ 2 induces cell apoptosis during liver regeneration has been unknown yet (11).

Controlling the expression of PLC $\gamma$ 2 in rat hepato-

cytes would be helpful in investigating its role in hepatocyte apoptosis. And the ideal tool for in vitro PLC $\gamma$ 2 delivery is adenoviral vector which has the obvious advantages comparing with other viruses, such as high gene delivery efficiency to various cells, large transgene capacity, the easy manipulation, high titer, no integration into the host genome and so on (12-14). Therefore, in our experiment, the recombinant adenovirus carrying PLC $\gamma$ 2 cDNA and report gene GFP was firstly packaged through co-transfecting the recombinant adenoviral shuttle vector pHBAAd-MCMV-GFP-PLC $\gamma$ 2 and backbone vector pHBAAd-BHG into HEK293 cell lines. The constructed recombinant adenovirus was used to infect rat normal liver line BRL-3A cells for evaluating transfection efficiency and primarily investigating its effect on apoptosis of rat liver cell line BRL-3A in vitro, which would lay the foundation for in-depth studying the action mechanism of PLC $\gamma$ 2 on hepatocyte apoptosis.

### Materials and Methods

#### Materials

Eukaryotic recombinant vector pHBAAd-MCMV-GFP-PLC $\gamma$ 2 has been constructed in our previous work. Human embryonic kidney cells (HEK293 cells) and Lipofiter™ transfection agent were obtained from Hanbio Co., Ltd (Shanghai, China). BRL-3A cells were gifted from Professor Cunshuan Xu (Henan Normal University, Henan,

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China). DMEM, fetal bovine serum (FBS) and trypsin were purchased from Hyclone Laboratories, Inc. (Logan, UT). Penicillin/Streptomycin and microfiltration membrane were purchased from Invitrogen (USA) and Millipore (USA), respectively. Plasmid DNA extraction kits and Gel Extraction Kit were from CWBIO company (Beijing, China) and Axygen (USA), respectively. Cell Cycle Analysis Kit and Annexin V-APC/7-AAD Apoptosis Detection Kit were purchased from KeyGEN BioTECH Corp., Ltd (Nanjing, China).

### Packaging and propagation of recombinant adenovirus vector

The recombinant shuttle vector pHBAd-MCMV-GFP-rat PLC $\gamma$ 2 constructed previously was extracted and purified according to the manufacturer's instructions (CWBIO, China). Meanwhile, HEK293 cells were grown in high glucose DMEM medium supplemented with 10% FBS, 1% penicillin/streptomycin at 37°C in a humidified incubator with 5% CO<sub>2</sub>. Once reaching a confluence of 70-80%, HEK293 cells were co-transfected with recombinant shuttle vector and backbone vector using Lipofiter™. After a figure "8" shaking motion, the mixture was incubated in DMEM containing 10% FBS at 37°C in a CO<sub>2</sub> incubator. Transfection medium was replaced with fresh medium at 6 hours after infection. Cell morphology was daily viewed until the appearance of cytopathic effect (CPE) characterized by cell swelling, rounding, bursting and then disappearance. The cells detached from the bottom of culture dish were transferred to Eppendorf tubes and underwent three cycles of freezing-thawing at temperatures between 37°C and -80°C. After centrifugation at 3000 rpm for 5 min, the supernatant was collected to get first-generation recombinant adenovirus (P1). The P1 virus was then continuously passaged twice in HEK293 cells to generate the P2, P3 viruses.

### Viral titer determination

The culture supernatant enriching third-generation adenovirus was collected for viral titer determination using tissue culture infectious dose 50 (TCID<sub>50</sub>) method. Briefly, the virus was serially diluted to graded concentrations of 10<sup>-6</sup>, 10<sup>-7</sup>, 10<sup>-8</sup>, 10<sup>-9</sup>, 10<sup>-10</sup>, 10<sup>-11</sup>, 10<sup>-12</sup>. The dilutions were used to infect HEK293 cells cultured in 96-well plates (10<sup>4</sup> cells per well) with 100  $\mu$ L DMEM containing 10% FBS. The cells were observed daily for CPE under light microscopy. Viral titer was

calculated according to the following format: virus titer (PFU/mL) = seeded cells  $\times$  optimum virus dilution  $\times$  10/virus volume added.

### Observation of virus infection by fluorescent microscope

Adenovirus at a multiplicity of 400 PFU/mL was infect into rat BRL-3A cells seeded in a 12-well plate (2 $\times$ 10<sup>5</sup> cells/well) containing DMEM supplemented with 10% FBS. After 24 h of transfection at 37°C in a humidified, 5% CO<sub>2</sub> incubator, GFP expression were inspected under fluorescent microscope.

### Quantitative real-time PCR (qRT-PCR) assay

BRL-3A cells were seeded into 12-well dishes at a density of 1 $\times$ 10<sup>5</sup> cells/well, and divided randomly into three groups including group I (no viruses for blank control), group II (Ad-GFP infection) and group III (Ad-PLC $\gamma$ 2 infection), 3 replicates per group. After twenty-four hours of culturing, the cells in each group were centrifugally harvested, respectively, and directly moved to Eppendorf tubes for quantifying rat PLC $\gamma$ 2 mRNA level by qRT-PCR method. qRT-PCR analysis was performed as follows: total RNAs were extracted from BRL-3A cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA), purified, and then reverse-transcribed to cDNA using a first-strand synthesis kit (Invitrogen, USA). The amount of cDNA concentration for PLC $\gamma$ 2 was quantified by real-time PCR. GAPDH was used as the internal control for normalization of data. qRT-PCR primer sequences to amplify PLC $\gamma$ 2 and GAPDH transcripts were designed based on the sequence of PLC $\gamma$ 2 and GAPDH genes using Primer 5.0 software (Table 2). After the data was normalized depending on the expression of GAPDH mRNA, rat PLC $\gamma$ 2 mRNA levels were calculated using the comparative Ct method ( $\Delta\Delta$ Ct Method). PCR reactions were done at least three independent replicates.

### Western blot analysis

BRL-3A cell line seeded in 12-well dishes were grouped as above. Twenty-four hours after culturing, cells were collected and lysed with ice-cold RIPA lysis buffer containing PMSF, followed by ultrasonic wave. Protein was centrifugally extracted, separated by 8% PAGE, and then transferred onto polyvinylidene fluoride (PVDF) membranes (Roche) at 100 mA for 2.5 h, soaked for 2 h in a blocking solution, and then immer-

**Table 1.** qRT-PCR assay results.

Groups	PLC $\gamma$ 2 Ct	GAPDH Ct	$\Delta$ Ct	$\Delta\Delta$ Ct
Control group	26.53667	17.15	9.38667	0.46
Ad-GFP group	27.48	18.55333	8.92667	0
Ad-PLCG2 group	11.66333	16.72333	-5.06	-13.98667

**Table 2.** qRT-PCR primer sequences of PLC $\gamma$ 2 and GAPDH.

Gene	Primer sequence (5'→3')
PLC $\gamma$ 2	FP: CTGGCAACCGACTCAAAGGA
	RP: GCTGATGCTGTTTCTTCGGG
GAPDH	FP: TTCCTACCCCAATGTGTCC
	RP: GGTCCTCAGTGTAGCCCAAG

sed in rabbit anti-rat PLC $\gamma$ 2 antibody diluent, or anti- $\beta$ -actin antibody used as a reference. After incubation for 24 h at 4°C, the membrane was washed in PBS buffer containing Tween 20, then incubated with HRP-labeled goat anti-rabbit IgG secondary antibody diluent for 30 min. The membranes were visualized by HRP chemiluminescent (ECL) detection system.

### Detection of cell apoptosis

Cell apoptosis was determined by flow cytometry (FCM) using an Annexin V-APC/7-AAD detection kit (NanJing JianCheng Bioengineering Institute, China). BRL-3A Cells were seeded onto six-well tissue culture plates at a density of  $2 \times 10^5$  cells/well and divided into three groups as mentioned above. The cells were infected with PBS buffer, empty adenoviruses Ad-GFP and recombinant adenoviruses Ad-PLC $\gamma$ 2 at a MOI of 400, respectively. Twenty-four hours later, cells were harvested by 0.25% trypsinization (without EDTA), washed with cooled PBS, and then centrifuged at 1500 rpm for 5 min. The harvested cell pellet was re-suspended in 50  $\mu$ L binding buffer and incubated with 5  $\mu$ L of 7-AAD. After incubation in the dark at room temperature for 15 min, the sample were again suspended in 450  $\mu$ L binding buffer and incubated with 1  $\mu$ L Annexin V-APC in the dark for 15 min. Cell apoptosis was analyzed by flow cytometry (BD Biosciences, Mountain View, CA, USA).

### Cell cycle analysis

Cells were plated in 6-well dishes ( $2 \times 10^5$  cells/well) and grouped as mentioned above. After infected with adenoviral vectors at a MOI of 400 for 24 hours, Cells were digested with by 0.25% EDTA-free trypsinization, washed twice with cooled PBS, and then centrifuged at 1500 rpm for 5 min. The harvested cell pellet was washed once with ice-cold PBS and then fixed with cooled 80% ethanol at 4°C for 4 h. After centrifugation (1000 rpm, 5 min), the cells were washed twice with ice-cold PBS and incubated with 100  $\mu$ L of RNase at 37°C for 30 min, then stained with 400  $\mu$ L PI for 30 min at 4°C in a dark environment. Cell cycle distribution was analyzed by a flow cytometer (BD Biosciences, Mountain View, CA, USA).

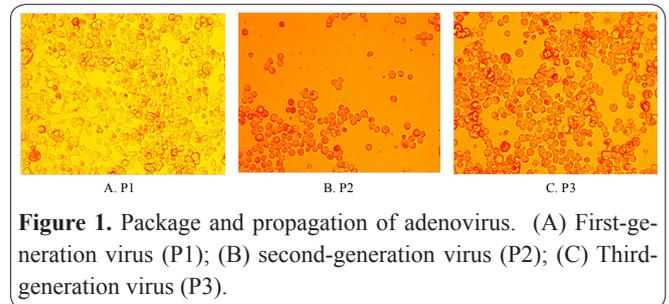
### Statistical analysis

Statistical analyses were performed using SPSS17.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as the means  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Statistically significant differences among more than two groups were performed using one-way analysis of variance (ANOVA). a  $P < 0.05$  were considered statistically significant.

## Results

### Packaging and propagation of Ad-PLC $\gamma$ 2 adenovirus

On the eleventh day after co-transfection by HBad-MCMV-GFP-PLC $\gamma$ 2 recombinant shuttle vector and pHBad-BHG backbone vector, the viral cytopathic effect emerged under light microscopy, and the adherent cells became rounded and ablated. After centrifugation, the harvested supernatant was the first-generation virus (P1 virus, Figure 1A). P1 virus was continuously pro-



**Figure 1.** Package and propagation of adenovirus. (A) First-generation virus (P1); (B) second-generation virus (P2); (C) Third-generation virus (P3).

pagated twice in HEK293 cells, thus generating P2, P3 viruses (Figure 1B, 1C). Obviously, cytopathic effect of viral infection was the most apparent after three-passage cultivation. Plaque assay showed that there was a titer of  $1 \times 10^{10}$  PFU/mL for the third-generation recombinant adenovirus, meeting the requirement for further transfection into BRL-3A cells.

### Fluorescence observation of GFP expression in the infected BRL-3A cells

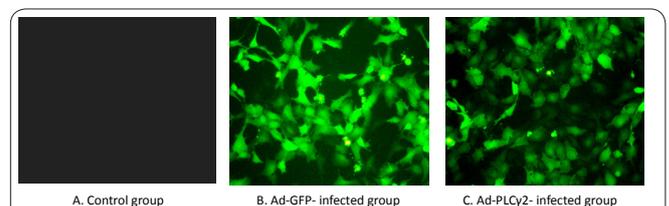
Twenty-four hours after infection, infection efficiency of Ad-PLC $\gamma$ 2 into BRL-3A cells was monitored by fluorescent signal detection under fluorescence microscopy. As shown in Figure 2, the non-infected blank control cells did not express fluorescence; a high percentage of fluorescence-expressing cells appeared in both Ad-GFP group and Ad-PLC $\gamma$ 2 group, but no morphological changes were observed. In addition, compared with the blank control, after Ad-PLC $\gamma$ 2 adenovirus infection at a MOI of 400, more than 90% of the cells were positive in GFP expression by inspecting under a fluorescent microscope, indicating that recombinant adenovirus effectively transfected BRL-3A cells, and did not damage to cell viability.

### Measurement of PLC $\gamma$ 2 mRNA level in BRL-3A cells by qRT-PCR

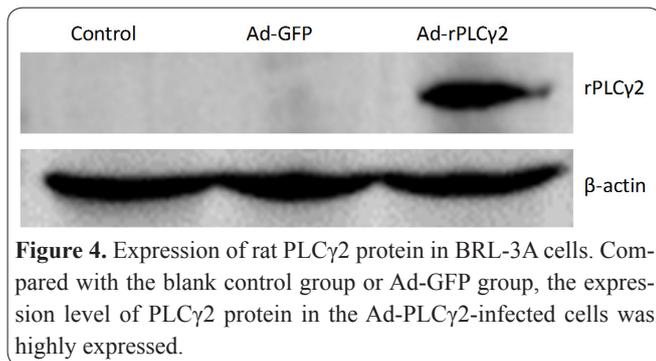
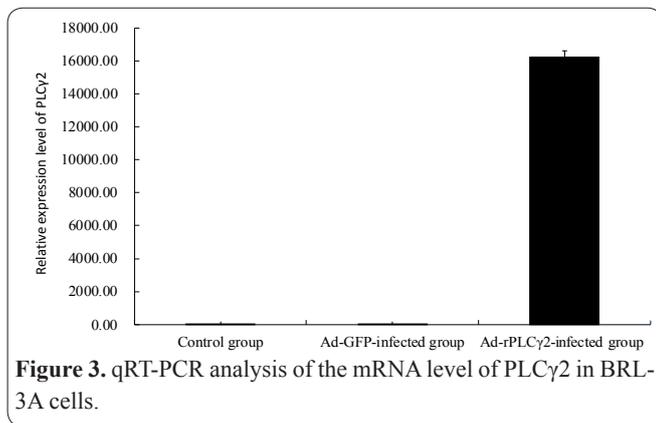
qRT-PCR results showed that, compared with the blank control (0.73) and Ad-GFP (1.01) groups, the relative mRNA level of PLC $\gamma$ 2 gene was significantly higher in the BRL-3A cells after 24 hours of infection with Ad-PLC $\gamma$ 2 (16241.88); whereas, there was no apparent difference in the mRNA level between Ad-GFP group and blank control group (Table 1 and Figure 3), suggesting that BRL-3A cells stably overexpressing PLC $\gamma$ 2 was constructed successfully via adenovirus vector.

### Western blot analysis of PLC $\gamma$ 2 expression level

At 24 hours after infection by the recombinant adenovirus Ad-PLC $\gamma$ 2 or empty adenovirus Ad-GFP, BRL-3A cells were harvested for western blot analysis. As shown in Figure 4, the expressions of PLC $\gamma$ 2 protein could not be detected in both blank control group and Ad-GFP



**Figure 2.** Observation of the green fluorescence in BRL-3A cells infected by null or recombinant adenovirus ( $\times 100$ ).



group, suggesting that BRL-3A cells themselves did not markedly express this protein. And in contrast to the cells in above two groups, PLC $\gamma$ 2 protein level in BRL-3A cells infected by Ad-PLC $\gamma$ 2 was increased markedly.

#### Effect of Ad-PLC $\gamma$ 2 adenovirus on apoptosis of BRL-3A rat liver cells

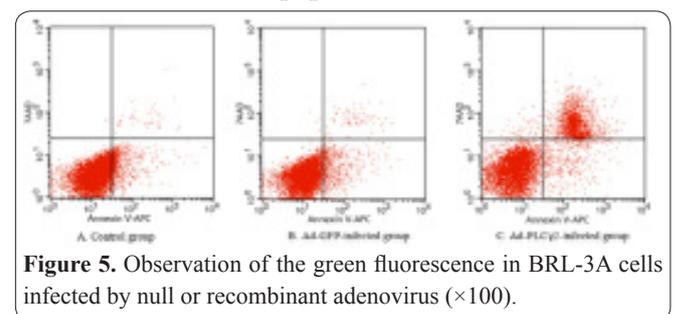
To define the effect of Ad-PLC $\gamma$ 2 on apoptosis in BRL-3A rat liver cells, the cells were analyzed by flow cytometry after 24 hours of infection. The results were as shown in Figure 5. Briefly, all the cells in the blank control group exhibited a low apoptosis rate of (6.68 $\pm$ 0.33)%. After Ad-GFP infection, the cell apoptosis rates increased slightly to (6.80 $\pm$ 1.06)%. Statistical analysis showed a nonsignificant difference ( $P > 0.05$ ) in cell apoptosis rates between control group and Ad-GFP infection group. whereas, post infection with Ad-PLC $\gamma$ 2, apoptosis rate in BRL-3A liver cells significantly increased to (26.15 $\pm$ 2.06)%. In the Ad-PLC $\gamma$ 2 infection group, the apoptosis rate was significantly higher (~4-fold,  $P < 0.01$ ) than those in control group and Ad-GFP infection group.

#### Effect of Ad-PLC $\gamma$ 2 adenovirus on the cell cycle of rat liver cell BRL-3A

To explore the possible mechanism underlying the proliferation-inhibitory effect of PLC $\gamma$ 2, in this study, the cell cycle distribution in BRL-3A cells after 24 hours of infection was analyzed by flow cytometry. The results are shown in Table 3 which displayed the percentage of distribution of cells in various cell cycle stages. Compared with those uninfected (control group) cells, BRL-3A cells infected with Ad-GFP demonstrated a slight increase in cell numbers at S stage ( $P > 0.05$ ), and a slight decrease in cell numbers at both G1 and G2/M phases ( $P > 0.05$ ). However, compared with the Ad-GFP infected cells, Ad-PLC $\gamma$ 2 infection led to a significant increase ( $P < 0.01$ ) in the cell percentage in G1 phase from 65.23% to 71.91%; a significant reduction ( $P < 0.01$ ) in percentage of cells in S phase from 22.58% to 18.46%, and a decline ( $P < 0.01$ ) in G2/M phase from 12.19% to 9.62%. Thus, Ad-PLC $\gamma$ 2 infection led to a decline in the S- and G2/M-phase cells, and an accumulation in G1-phase cells. These results indicate that PLC $\gamma$ 2 over-expression arrests the cell cycle at the G1-S boundary.

#### Discussion

PLC $\gamma$ 2, a key enzyme of phosphoinositide signal transduction pathway, is widely located in various cell types. When activated, this enzyme hydrolyzes PIP<sub>2</sub> into DAG and IP<sub>3</sub>. The former could activate typical PKC-mediated signaling pathway, and the latter could stimulate Ca<sup>2+</sup> signaling by causes the mobilization of Ca<sup>2+</sup> from internal stores (15,16), subsequently participating in regulation of a large array of cellular processes, such as cell proliferation and differentiation (17). As the proceeding of the related investigation, in recent years, several evidences indicated that PLC $\gamma$ 2 also played the vital role in regulating cell apoptosis. For instance, Takata and his colleagues observed that the induction of cell apoptosis was blocked in PLC $\gamma$ 2-



**Table 3.** Flow cytometry analysis of cell cycle effects of Ad-PLC $\gamma$ 2 on BRL-3A cells (%).

Groups	Cell cycle		
	G1 phase	S phase	G2/M phase
Control group	65.26 $\pm$ 0.96 <sup>aA</sup>	21.26 $\pm$ 0.17 <sup>aA</sup>	13.47 $\pm$ 1.11 <sup>aA</sup>
Ad-GFP- infected group	65.23 $\pm$ 0.66 <sup>aA</sup>	22.58 $\pm$ 1.42 <sup>aA</sup>	12.19 $\pm$ 0.76 <sup>aA</sup>
Ad-PLC $\gamma$ 2- infected group	71.91 $\pm$ 0.44 <sup>B</sup>	18.46 $\pm$ 0.53 <sup>B</sup>	9.62 $\pm$ 0.34 <sup>B</sup>

Same small letters within the same column denote no significant difference ( $P > 0.05$ ); the different capital letters denote significant difference ( $P < 0.05$ ).

deficient DT40 cells, suggesting PLC $\gamma$ 2 were essential for cell apoptosis (18). Zhang *et al.* (2003) also found that PLC $\gamma$ 2 gene was significantly up-regulated during TPA-induced apoptosis in gastric cancer cells, implying a critical role of this messenger in apoptosis induction (10). However, to date, little is known about whether PLC $\gamma$ 2 is implicated in the regulation of hepatocyte apoptosis during liver regeneration.

Transfer of genes into the target cells is critical for exploring gene function (19). By far, gene-delivering vectors mainly include viral vectors and nonviral vectors (20). The former are represented by replication-deficient, genetically modified retrovirus or adenovirus. Among viral-based vector system, adenovirus is the most widely used due to its many superiorities like high titer and the easily manipulated virus genome (21,22). Nonviral vectors, mainly including liposome and plasmids, can flexibly deliver various sizes of genes into targeted cells, but this gene delivery tool has the relatively low delivery efficiency (23,24). Therefore, in order to probe into the role of PLC $\gamma$ 2 in regulating hepatocyte apoptosis during liver regeneration, this study constructed the recombinant adenovirus vector carrying rat PLC $\gamma$ 2 using AdMax adenoviral vector system, and transfected Ad-PLC $\gamma$ 2 into rat BRL-3A cells—one stable liver cell line similar to rat hepatocyte in the physiological and biochemical characteristics.

In the present study, the recombinant shuttle vector pHBAd-MCMV-GFP-PLC $\gamma$ 2 used for packaging of the recombinant adenovirus had been already prepared in our previous work. In this shuttle vector, multiple cloning sites (MCS) for connecting the target gene and green fluorescent protein (GFP) gene share the same promoter--CMV who allows for the simultaneous transcription of the target gene and GFP once cloning the target gene into MCS (25). Of note, GFP serves as one reporting molecule enabling the detection of target gene expression in living cell because it is easily observable under fluorescence microscopy (26,27). In this study, the recombinant shuttle vector and backbone vector were co-transfected into in HEK293 cells for virus packaging. After 10 days of viral cultivation, the typical cytopathic effect was observed under microscope. After three passages, the infectious viral titer was high up to  $1 \times 10^{10}$  PFU/mL, which met the demands for the subsequent experiments.

After 24 hours of transfection of the constructed viral vector (Ad-PLC $\gamma$ 2) and empty viral vector (Ad-GFP) into BRL-3A cells, respectively, comparing with blank control unexpressing GFP, green fluorescence was observed in >90% of the living cells in above two groups were observable under fluorescent microscope, but cellular morphology was not altered, which demonstrated a successful transfection of viral vector into BRL-3A cells, but no damage to the target cells. To detect the expression of PLC $\gamma$ 2, the total RNAs was extracted from BRL-3A cells for qRT-PCR assay. Results showed that mRNA level of PLC $\gamma$ 2 gene in Ad-PLC $\gamma$ 2 group was significantly increased by >16000-fold higher than that the blank control and Ad-GFP groups. This indicated that adenovirus vector enabled the target cell to over-express PLC $\gamma$ 2 gene. To further confirm the over-expression of PLC $\gamma$ 2 in BRL-3A cells infected by recombinant adenovirus, PLC $\gamma$ 2 protein level was evaluated

by western blot. Results showed that, on the third day of transfection, the recombinant adenovirus vector significantly increased the expression of PLC $\gamma$ 2 protein in BRL-3A cells, reinforcing the conclusion drawn from qRT-PCR data. According to above results, it was inferred that recombinant adenovirus Ad-PLC $\gamma$ 2 was successfully constructed.

As described previously, PLC $\gamma$ 2 has been documented to have an important role in regulation of cell apoptosis (10). For instance, up-regulated expression of PLC $\gamma$ 2 could inhibit cell growth and induce cell apoptotic death, and deletion of PLC $\gamma$ 2 gene always blocked apoptotic cell death of T-cell (18). To define the influence of PLC $\gamma$ 2 on apoptosis of liver cell BRL-3A which is similar to the biological characteristics of rat hepatocyte, cell apoptotic rate was analyzed using a flow cytometer. The results showed that Ad-PLC $\gamma$ 2 infection cause a significant increase ( $P < 0.01$ ) in cell apoptotic rate in contrast to those cells from blank control and empty adenovirus groups, demonstrating that PLC $\gamma$ 2 overexpression significantly induced apoptosis in BRL-3A cells *in vitro*. Our results are supported by the findings of previous studies that PLC $\gamma$ 2 exhibited apoptosis-promoting effects in benign and malignant cells (10,18). These data provided the evidence that PLC $\gamma$ 2 acts as a positive regulator for BRL-3A cell apoptosis. Next, to explore the action mechanism of PLC $\gamma$ 2-induced cell apoptosis, we primarily detect cell cycle distribution of BRL-3A cells infected with Ad-PLC $\gamma$ 2. The results showed that, compared with the uninfected cells and Ad-GFP infected cells, Ad-PLC $\gamma$ 2 infection led to a significant increase ( $P < 0.01$ ) in cell percentage in G1 phase, meanwhile a significant decrease in percentage of cells in S phase and G2/M phase ( $P < 0.01$ ), demonstrating that PLC $\gamma$ 2 could induced cell cycle arrest at G1 phase in BRL-3A cells. Above results revealed that PLC $\gamma$ 2 could promote cell apoptosis by arresting cell cycle at G1 phase.

In brief, to our knowledge, this is the first study to explore the possible role and mechanisms of PLC $\gamma$ 2 in rat liver cells. Our results showed the successful establishment of recombinant adenovirus Ad-PLC $\gamma$ 2 and the pro-apoptosis effect of PLC $\gamma$ 2 on rat liver cells through causing cell-cycle arrest in G1 phase. However, it is still unclear how PLC $\gamma$ 2 induces cell apoptotic death. In future, we would be devoted to clarify the exact molecular mechanism of rat PLC $\gamma$ 2 gene in inducing the apoptosis in rat hepatocytes *in vitro*.

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