Establishment and identification of HeLa cell lines with stable expression of APOL1

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Abstract

Objective: Apolipoprotein L1 (APOL1) is an interferon-inducible protein with a wide range of innate immune functions. Studies have shown that APOL1 can restrict the replication of viruses (including HIV) in vitro. However， the molecular mechanism of APOL in restriction the viral replication and association with immune pathways has remained unclear.Thus, we establish a HeLa cell lines that stably and efficiently expresses APOL1 for studying the molecular mechanism of APOL1 and the immune system in vitro. Methods: To generate lentiviral particles, the recombinant lentiviral vector pCDH-APOL1 was transfected into 293T cells by Lipofectamine 2000. After recombinant lentivirus infection, the culture was screened with puromycin to obtain HeLa cells stably expressing APOL1. Finally, the expression of APOL1 in the constructed HeLa cell line was identified by Western blotting. Results and Conclusion: The results indicated that a HeLa cell lines stably expressing APOL1 was successfully established by a lentivirus-mediated expression system. It is important to study the relationship between APOL1 and immune signaling pathway.

Keywords

APOL1, lentiviral, over-expression, HeLa cells.

1. Introduction

As the product of a member of a family of *APOL* genes, apolipoprotein L1 (APOL1) is a component of the innate immune system[1, 2]. By analyzing the *APOL1* gene location on the human chromosome, we can be surprised to find it is located in the vicinity of a cluster of restriction factor *APOBEC3* genes known to control the expression of endogenous retroviruses[3]. It has been recently shown that APOL1 are likely associated with innate immune responses. On the one hand, in human macrophages infected with HIV-1, overexpression of APOL1 inhibits the expression of the viral key proteins Gag p55 and p24[4]. Endogenous expression of APOL1 in differentiated U937 monocytic cells stimulated with IFN-γ resulted in a reduced production of HIV virus particles[3]. On the other hand, in human monocyte THP-1 cells, transient overexpression of APOL1 by transfection increase in CD14 and CD68 gene expression. Interestingly, APOL1 is also caused monocytes to differentiate into atypical M1 macrophages with marked increase in M1 markers CD80, TNF, IL1B, and IL6 and modest increase in the M2 marker CD163[5]. These reports suggest that the APOL1, in addition to having evolved to play an anti-parasite role, also have anti-viral roles and has a close relationship with the innate immune process. In the present study, we constructed HeLa cell lines stably overexpression APOL1 by lentiviral-mediated and to lay the foundation for further study of the role of APOL1 in the innate immune process.

2. Materials and Methods

2.1 Materials

Lentiviral plasmid vector pCDH, pCDH-RFP (positive control), lentiviral packaging plasmid psPAX2 and PMD2.G were purchased from Addgene; human cell lines 293T and HeLa were maintained using standard protocols from ATCC.

2.2 Cell culture

293T and HeLa cells were cultured in high-glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. Cells were maintained at 37 °C and in a 5% CO_2 atmosphere incubator.

2.3 Amplification of target gene

The primers were designed based on the *APOL1* gene sequence provided in NCBI GenBank (Gene ID: 8542). HeLa cells were treated with 1000 U/mL IFN-γ for 24 hours, and total RNA was extracted. The total RNA was reverse transcribed into cDNA, and the fragments APOL1 was amplified with the cDNA as template. The cycling parameters of PCR were as follows: pre-denaturation at 95 for10min, then 35 cycles of denaturation at 95°C for 30 s, annealing with TM value of 58°C for 30 s and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min. PCR products were identified by 1% agarose gel electrophoresis. The sequences of primers for PCR were as follows:

Forward:5'-GAATTCATGGAGGGAGCTGCTT-3' ;

Reverse:5'-GGATCCATACAGTTCTTGGTCCG-3'.

2.4 Construction and identification of the recombinant lentiviral vector

The pCDH vector and the PCR product were digested with restriction enzymes EcoRI and BamHI, and the digested product was purified and transformed into DH5α competent cells after ligation at 25°C for 2 hours. Four clones were randomly selected, inoculated into LB liquid medium containing ampicillin, and cultured overnight at 37°C in an incubator with shaking at 250 rpm/min. The presence of the target gene was confirmed by colony PCR identification and double enzyme digestion, and the positive clone was subjected to Sanger sequencing.

2.5 Lentiviral production and concentration

293T and HeLa cells were cultured in high-glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 mg/mL streptomycin. Cells were maintained at 37 °C and in a 5% CO_2 atmosphere incubator. The recombinant lentiviral vector pCDH-APOL1 (3μg) and pCDH-RFP (3μg) was respectively co-transfected with 3μg psPAX2 and 3μg pMD2.G into 293T cells, according to the instructions of Lipofectamine 2000 (Invitrogen, USA) Reagent. The supernatant containing viral particles was harvested twice at 48 and 72 h after transfection, and the cell debris was removed by centrifuged at 4000 rpm/min.

2.6 Lentivirus infection and establishment HeLa cell lines with stable overexpression of APOL1

HeLa cells were plated in 6 well dishes at 4×10^5 cells per well. The next day, cells were infected with the lentivirus with 8 μg/mL polybren. After 24 hours, the cells infected with the lentivirus carrying the pCDH-RFP vector were observed red fluorescence, media was replaced with media containing 2 μg/mL puromycin. To increase overexpression efficiency, infected cells were under 10-15 days of puromycin selection. Overexpression efficiency of proteins was evaluated by WB.

2.7 Western blot analysis

Total protein was extracted from cells using Western and IP cell lysate (Beyotime Biotechnology, China), and loaded and separated on a 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel, and subsequently transferred onto PVDF membrane (Millipore, USA). The PVDF membranes were blocked with TBS-T containing 5% nonfat milk for 1 h at room temperature, then incubated respectively with primary antibodies: anti-APOL1 antibody (ProteinTech, USA; 1:2000), anti-

internal reference Tubulin antibody (ProteinTech, USA; 1:2000) at 4°C overnight. Following washing in TBS-T, the membranes were probed with HRP-conjugated secondary antibodies (ProteinTech, USA; 1:5000) for 2 h at room temperature and immunoblots were visualized using an ECL substrate kit (Millipore, USA).

3. Results

3.1 Construction and identification of overexpression APOL1 recombinant lentivirus vector

APOL1 was reported as an IFN-γ stimulating gene, and APOL1 was effectively induced to express when interferon stimulating agents were used[6]. HeLa cells were treated with 1000 U/mL IFN-γ for 24 h, and total RNA was extracted. The total RNA was reverse transcribed into cDNA, and the fragments APOL1 was amplified with the cDNA as template. The APOL1 gene product (Fig. 2 A) were obtained by PCR with previously extracted cDNA as template. PCR products underwent agarose gel electrophoresis, and the bands size was consistent with the expectation. The APOL1 gene products were separately ligated with the pCDH vector (Fig. 1 A), and transformed into DH5α competent cells after ligation .The selected 4 clones were identified by colony PCR, and the results (Fig. 2 B) showed that clone 1 and clone 2 showed a target band at the corresponding size position. Finally, we identified the clone 1 by digestion with EcoRI and BamHI (Fig. 2 C), and the clone 1 has generate two bands at the corresponding sites. The clone 1 was subjected to Sanger sequencing, and the sequencing results were analyzed by a BLAST search. The results showed that both the inserted sequence and the read-through box were correct and the plasmid was constructed successfully.

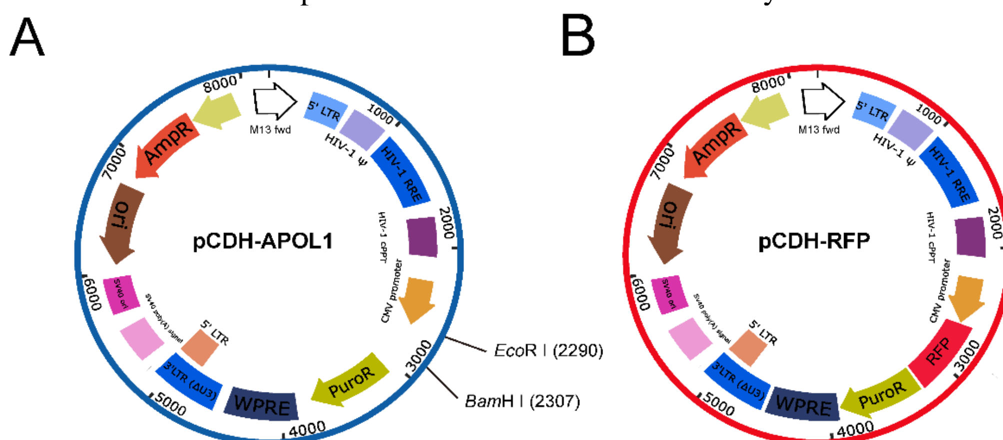

Fig. 1 Map of pCDH-APOL1 and pCDH-RFP Lentivirus Interference Vector

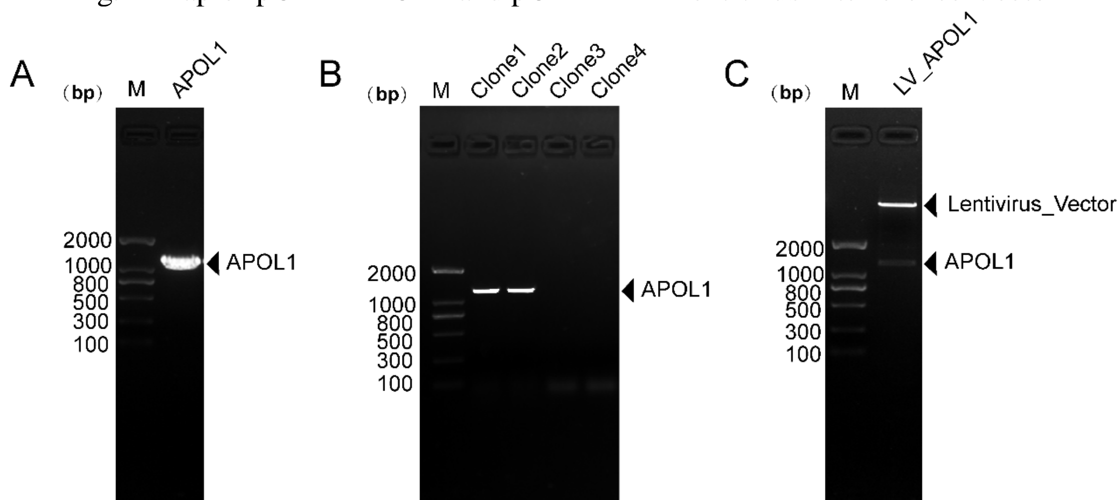

Fig. 2 Construction and identification of the recombinant lentiviral vector

(A) PCR amplification product of APOL1; (B) Identification of recombinant lentiviral vector by colony PCR; (C) Identification of a recombinant lentiviral vector by EcoRI and BamHI.

3.2 Establishment of the cell line with stable overexpression of exogenous protein

The recombinant lentiviral vector pCDH-RFP (Fig. 1 B and Fig. 3) was used as a positive control during lentiviral packaging and infection. As shown in Figure 3, red fluorescence was clearly observed when pCDH-RFP was transfected into 293T cells, indicating that the lentivirus has been successfully packaged. When Hela cells infected with lentivirus carrying pCDH-RFP, red fluorescence can be clearly seen under fluorescence microscopy after 24 h. Whereas in the groups of Hela cells infected with lentivirus carrying pCDH-RFP, red fluorescence was not observed. Collectively, these findings reveal that the lentivirus using in this experiment was successfully packaged and can efficiently infected the HeLa cells.

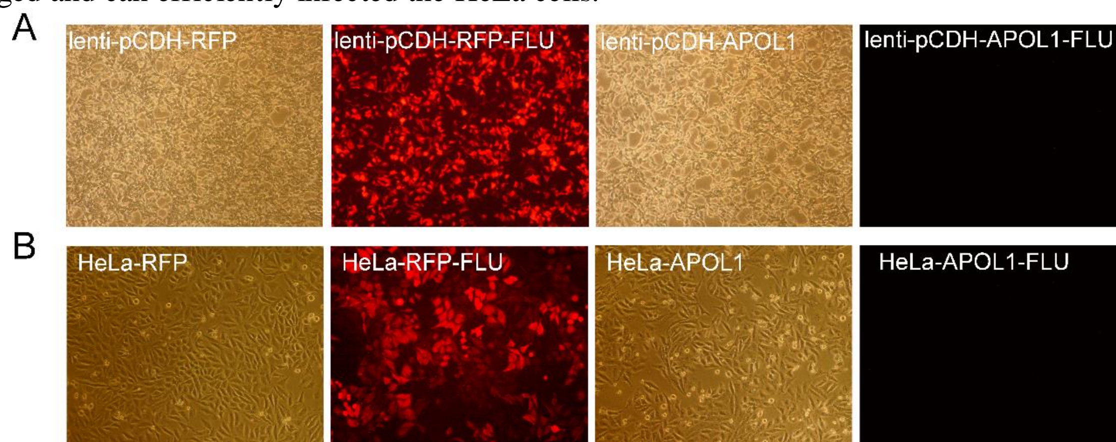

Fig. 3 Targeting packaging and infection of pCDH-APOL1 lentivirus（×100）
(A) Packages targeting pCDH-APOL1 lentiviruses; (B) Targeting pCDH-APOL1 lentivirus successfully infected HeLa cells.

3.3 Western blotting detection of APOL1 in HeLa cells expression

APOL1 protein levels were determined by Western blot and compared to control cells (HeLa and HeLa-RFP). We found that APOL1 expression levels (Fig. 4) were significantly upregulated in stable overexpression APOL1 HeLa cell lines (HeLa-APOL1). Taking together, these results further demonstrated that promotion of APOL1 expression by lentivirus-APOL1 was effective at protein level.

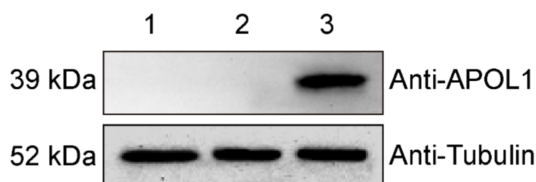

Fig. 4 Western blotting analysis of APOL1 expression in HeLa cells
(1. HeLa cells; 2. HeLa-RFP cells; 3. HeLa-APOL1 cells.)

4. Discussion

APOL1 as a minor protein component of high density lipoprotein, and its expression is driven by innate immune factors such as interferon and tumor necrosis factor (TNF)[7]. Previous research shows, the APOL1 promoter contains regulatory elements for the transcription factors STAT2, interferon regulating factor (IRF) 1 and (IRF) 2, suggesting that APOL1 is a cellular immune response gene, whose expression is stimulated by viral infection[8]. At the same time, some studies have shown interferon seems to be a strong regulator of APOL1 expression and in human subjects STAT1 shows a remarkable correlation with APOL1 levels[9]. Taken together, these studies suggest that APOL1 acts as an innate immune factor and plays an important role in the immune system and antiviral processes.

Generally, plasmid transfection is transiently transfected with low efficiency, loss and silencing, and the target gene is rarely integrated into the genome after transfection[10]. The lentiviral vector is

developed on the basis of HIV-1, which can efficiently infect various types of cells, integrate the viral genome into the host genome, and stably express exogenous genes for a long time[11]. Lentivirus has a high safety and is a powerful gene transport tool, which has gradually become a common plasmid vector in gene research.

In this study, we successfully established Hela cell lines stably expressing APOL1 by a lentivirus-mediated expression system. This will lay a solid foundation for the subsequent study of the relevant molecular mechanisms of APOL1 in the innate immune process.

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