Original Article

Effect of apolipoprotein B mRNA-editing catalytic polypeptide-like protein-3G in cervical cancer

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Abstract: Cervical cancer is one of the most common gynecologic cancers. The role of apolipoprotein B mRNA-editing catalytic polypeptide-like protein-3G (APCBEC-3G) in cervical cancer has yet to be elucidated. This study intends to explore the effect of APCBEC-3G on cervical cancer cell proliferation and invasion. In vitro, the cervical cancer cell line Hela was transfected by APCBEC-3G plasmid. The mRNA and protein expression levels of APCBEC-3G were detected by Real-time PCR and Western blot, respectively. Cervical cancer cell proliferation was determined by MTT. Transwell assay was applied to measure the effect of APCBEC-3G on cell invasion. APCBEC-3G mRNA and protein increased significantly after transfection (P<0.05) and cervical cancer cell proliferation and invasive ability were decreased significantly (P<0.05). APCBEC-3G serves as a suppressor of cervical cancer cell proliferation and invasion. Our research provides theoretical basis for further investigation of APCBEC-3G effect in cervical cancer occurrence and development.

Keywords: APCBEC-3G, cervical cancer, proliferation, invasion

Introduction

Cervical cancer is common among gynecologic malignant tumors and accounts for the second incidence in women worldwide [1] and in China, it presents the top incidence in women cancer and increasingly younger [2]. Persistent infection with high-risk types of human papillomavirus (HPV) is considered a potent factor closely associated with the onset of the cervical cancer [3]. Though patients’ survival and quality of life have been greatly improved following the medical development, the morbidity and mortality of cervical cancer have not yet decreased obviously [4, 5].

Apolipoprotein B mRNA-editing catalytic polypeptide-like protein-3 (APOBEC-3) belongs to the family of DNA cytosine deaminases that is unique in mammals with cytidine deaminase activity. It plays roles in catalyzing peptide protein, transforming cytosine deamination to uracil, and generating mutations in cells. It’s characterized as a DNA-RNA editing enzyme in the cell [6, 7]. Study suggested that APOBEC-3 acts as an important cytokine in innate immune response against exogenous infection especially viral infection [8]. APOBEC-3 has been proposed an inhibiting role in viral infection such as parvovirus, adenovirus, HIV-1 [9, 10]. It edits HPV DAN in nucleus of HPV infected cells to eliminate HPV infection. Therefore, APOBEC-3 may serve as the potential function in the occurrence and development of cervical cancer [11]. APOBEC-3 family includes APCBEC-3A, APCBEC-3B, APCBEC-3C, APCBEC-3D, APCBEC-3F, and APCBEC-3H [12], among which, APCBEC-3A and APCBEC-3C have been demonstrated to enhance the recognition of human HPV infection and to be of importance for the susceptibility of to the occurrence of cervical cancer [13]. Recently discovery showed APOBEC-3G, as a new member in APOBEC-3 family, inhibited vif defective HIV-1 [14]. Besides, it also blocked the replication of multiple viruses such as parvovirus and HBV DNA viruses [15]. However, the role of APOBEC-3G in cervical cancer has so far remained elusive. Our study aims to investigate...
apoBEC-3G in cervical cancer

the effect of apoBEC-3G on cervical cancer cell proliferation and invasion.

Materials and methods

Main reagents and instruments

Hela cell line was obtained from ATCC cell bank. ApoBEC-3G and control plasmids were got from Fulengen (Guangzhou, China). ApoBEC-3G polyclonal antibody was from Abcam. Trizol reagent was from Life technology, DMEM medium, fetal bovine serum (FBS), and penicillin-streptomycin were from Hyclone. Dimethyl sulfoxide and MTT were purchased from Gibco. Trypsin-EDTA was from Sigma. PVDF membrane was got from Pall Life Sciences. Western blot related chemical reagents were from Shanghai Beyotime biotechnology co., LTD. ECL reagent was from Amersham Biosciences. β-actin primary antibody and HRP tagged IgG secondary antibody were from Cell Signaling. Plasmid extraction kit and E.coli DH-5α were from Takara co., LTD. Other reagents were purchased from Shanghai Sangon biotechnology co., LTD. Labsystem Version1.3.1 microplate reader was purchased from Bio-rad.

Hela cell culture and grouping

Hela cells were maintained with high glucose DMEM medium (containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin) at 37°C and 5% CO2. The medium was changed on the other day and cells were passaged every 2-3 days. The cells were randomly divided into three groups, including control group, blank group, and transfection group.

ApoBEC-3G transfection

pcDNA3.1-apoBEC-3G plasmid was amplified in E.coli DH-5α and extracted using the plasmid extraction kit. The pcDNA3.1-apoBEC-3G plasmid was then concentrated and quantified as concentration at 1 μg/μl and A260/A280 = 1.8. Next, the plasmid was transient transfected into Hela cell by using the lipofectamine 2000 reagent. Specially, 2 μg pcDNA3.1-apoBEC-3G plasmid and 5 μl lipofectamine were dissolved in 250 μl DMEM medium for 20 min at room temperature. Then they were added to Hela and incubated at 37°C for 24 h.

Real-time PCR

The cDNA was synthesized with 1 μg RNA from the samples. The primers are used as follows. ApoBEC-3G: forward primer: 5’-CTCAGCGGAT-
PCR amplifications were performed in three duplicates for each sample. Gene expression levels were quantified relative to the expression of GAPDH using an optimized comparative Ct (ΔΔCt) value method.

Western blot

The tissue was digested with lysis buffer. Total protein was separated by denaturing 10% SDS-polyacrylamide gel electrophoresis. Detection was performed with chemiluminescence and calculated with Quantity One. Antibody dilutions were 1:500 for MMP-8 and TIMP. Protein levels were normalized to β-actin and changes were determined by four times replicates.

MTT assay

After cultured for 24 h, 48 h, and 72 h, the cells in each group were digested and counted. And then, the cells were seeded to the 96-well plate at 3000 cells/well with five replicates. 20 μl 5 g/L MTT solution was added to each well and cultured at 37°C and 5% CO₂ for 4 h. After adding 150 μl DMSO for 10 min, the absorbance (OD) value of each well was detected by microplate reader at wavelength of 570 nm to calculate the cell proliferation rate.

Transwell assay

300 μg/ml Matrigel was added to the surface of upper chamber at 4°C under sterile condition and incubated at 37°C for 3 h. DMEM medium containing 15% FBS was added to the lower chamber. The cells were pretreated with

CTAAACGGAAT-3', reverse primer: 5'-CACATTCT-GGCGTTCCGTA-3'; GAPDH: forward primer: 5'-AGTCTAGTTGCTGG-GTACC-3', reverse primer: 5'-TAAATAGGTGTCTGGACTCGG-3'. The cycling conditions consisted of an initial, single cycle of 1 min at 52°C, followed by 35 cycles of 30 s at 90°C, 50 s at 58°C, and 35 s at 72°C.
FBS free DMEM medium for 12 h and then seeded in the upper chamber. After 20 h incubation, the membrane was fixed in 70% formaldehyde for 30 min. Crystal violet was used for staining and the cells were counted under the microscope.

Statistical analysis

All statistical analyses were performed using SPSS16.0 software (Chicago, IL). Numerical data were presented as means and standard deviation (± S). Differences between multiple groups were analyzed using one-ANOVA. $P<0.05$ was considered as significant difference.

Results

**APOBEC-3G mRNA expression in Hela**

APOBEC-3G mRNA expression was detected by real-time PCR after APOBEC-3G plasmid transfection for 24 h. The level of APOBEC-3G mRNA was significantly higher in Hela cell transfected with APOBEC-3G plasmid compared with that in control and empty plasmid group ($P<0.01$) (Figure 1).

**APOBEC-3G protein expression in Hela**

Western blot was applied to determine APOBEC-3G protein expression changes in Hela cells. These findings were consistent with the results of APOBEC-3G mRNA expression, APOBEC-3G protein was up-regulated markedly after APOBEC-3G transfection ($P<0.01$) (Figures 2 and 3). These results identified that APOBEC-3G was successfully transfected to Hela cells.

**Impact of APOBEC-3G on Hela cell proliferation**

Hela cell proliferation was determined by MTT method. The cell proliferation of Hela
decreased obviously after APOBEC-3G transfection compared with control (P<0.05), and this inhibitory effect showed time-dependent (Figure 4).

**Impact of APOBEC-3G on Hela cell invasion**

Transwell assay was performed to detect the impact of APOBEC-3G on Hela cell invasion. The transmembrane cell number in APOBEC-3G group was significantly lower than that in control and empty plasmid group (P<0.01) (Figures 5 and 6). These data indicated that APOBEC-3G could inhibit Hela cell invasion.

**Discussion**

The innate immune response is the first defensive step to viral infection. APOBEC plays an essential role in the innate immune response against viral infections. Recent discovery demonstrated that as one of most important components in innate immune reaction, APOBEC-3G mainly distributes in T cells, monocytes, and macrophages [16]. Studies also illustrated that APOBEC-3G inhibits replication, of retroviruses, such as mouse leukemia virus (MLV), human T-cell lymphotropic virus, parvovirus, hepatitis C virus (HCV), etc. It interferes viral DNA, DNA nucleocapsid and RNA packaging to suppress HBV replication. APOBEC-3G contributes cytosine deamination effect to limit HIV-1 virus replication. Therefore, it serves as an inhibitor to treat a variety of virus infection diseases [17-19].

Cervical cancer is closely associated with high-risk HPV persistent infection. High rates of persistence of HPV have been reported in immuno-compromised women and the permissiveness of cervical epithelial cells to HPV infection increases the risk of cervical cancer [20]. Some APOBEC-3 family members, such as APOBEC-3A and APOBEC-3C, have been validated to be able to regulate HPV infection [13, 21]. However, the effect of APOBEC-3G on cervical cancer remains to be further elucidated. Our results indicated that the level of APOBEC-3G mRNA and protein expression significantly increased after its transfection into Hela cells. APOBEC-3G overexpression overwhelmingly inhibits proliferation and invasion of cervical cancer cells. APOBEC-3G is a host cytidine deaminase that possesses an antiviral role in human innate immune system. Recent studies on the role of APOBEC-3G in different infectious diseases and virus-associated cancers offer clinical support to therapeutic targets selection. Our study illuminates an essential inhibitory role of APOBEC-3G in proliferation and invasion of cervical cancer cells, and provides theoretical basis for further investigation of APOBEC-3G in cervical cancer occurrence and development.

**Disclosure of conflict of interest**

None.

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