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Original Article

Effect of DEK gene silencing on proliferation and apoptosis in human oral squamous cell carcinoma PCI-37 B cells

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Abstract: Oral squamous cell carcinoma (OSCC) is one the oral diseases of which are major causes of cancer-related death. Despite the development of advanced technologies in clinical and experimental oncology in recent years, the 5-year survival rate is still low. Therefore the identification of novel OSCC related molecules and the discovery of new makers and drug targets are essential. The human DEK gene has been implicated as an oncogene in OSCC, which has been proved to participate in several critical biological signaling pathways including the expression of protein and mRNA. This study demonstrates that DEK is highly expressed in OSCC cells compared to normal tissue cells. Additionally, inhibition of DEK gene expression can effectively inhibit the proliferation of oral cancer cells. Also the inhibition of DEK gene arrest cells in G0/G1 phase and then impact proliferation and differentiation, thus suggesting that DEK may participate in the procedure of OSCC growth and progression.

Keywords: DEK, oral squamous cell carcinoma, gene silencing, proliferation, apoptosis

Introduction

Oral squamous cell carcinoma (OSCC) is a malignant tumor of oral maxillofacial region with high incidence rate. Despite advanced technologies has been developed in both clinical and experimental fields in recent years, the prognosis is still unfavorable due to its invasive characteristics and highly malignancy, the 5-year survival rates remain at less than 50% and have not been improved in the last 3 decade. Therefore, the traditional treatment method has been unable to meet the needs of the patients and new therapeutic strategies must be evaluated to improve the prognosis of OSCC patients. More and more scholars focus on the pathogenesis of tumor targeted therapy and gene research, from the molecular level research that the role of genes involved in tumorigenesis and metastasis to molecular mechanism, select targeted genes. Hence, it is vital to uncover the biological mechanisms of the disease for the sake of identifying useful biomarkers and novel therapeutic targets.

DEK was originally discovered in patients with a subtype of acute myelogenous leukemia as a fusion product in which the 26 C-terminal amino acids are replaced by the N-terminal two-thirds of the nucleoporin CAN, which has also been termed NUP 214 [1-4]. Since its discovery, DEK has also been found to be transcriptionally upregulated in various aggressive human tumors such as retinoblastoma, hepatocellular carcinoma, colon cancer, bladder cancer, cervical cancer, malignant glioma, melanoma, and gastric cancer [5-16]. Therefore DEK has been identified as being an oncogene [17], because of its two-fold role both promoting and inhibiting of apoptosis, it plays very important regulating roles along with other cell apoptosis related factors in the cell proliferation and differentiation, apoptosis, and participates in the occurrence and progression of cancer. As for DEK

protein regulation of cell apoptosis is antiapoptotic effect or its effect on promoting apoptosis, may be related to DEK protein affected by specific regulatory factors related to cell apoptosis.

The previous research found that the expression of DEK mRNA and DEK protein were higher in oral squamous cell cancer and metastasis lymph node tissue than that in tissue adjacent to carcinoma, it prompted that DEK oncogene played a significant role in the occurrence and metastasis of oral squamous cell carcinoma [18]. Purpose of this study is to investigate the impact of DEK gene siRNA in PCI-37B cells and its biological mechanism, to support the diagnosis and treatment of OSCC in theoretical basis as well as to reveal the effect of gene targeting therapy and provides us a promising avenue for cancer therapy.

Materials and methods

Cell lines and cell culture

Metastatic oral squamous cell carcinoma cell lines PCI-37B were obtained from University of Pittsburgh Medical Center. PCI-37B cells were cultured in DMEM supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin, and 100 μg/mL streptomycin. All the cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

Transient transfection assay

1×10^4 cells were plated onto 6-well plates and grown to 30-50% confluence before transfection. Transfection of cells was performed using Oligofectamine (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

Detection of cell proliferation by MTT assay

Forty-eight hours after transfection, OSCC cells were harvested, seeded into 6-well culture plates at a density of 1×10^4 cells in 100 μL/well, and incubated at 37°C. At different time points (24, 48, or 72 h), 20 μL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for another 3 h at 37C. Then, the MTT solution was removed and 150 μL dimethyl sulfoxide (DMSO) was added to each well to stop the reaction. The plates were gently shaken on a swing bed for 10 min, and spectrometric absorbance at 490 nm was measured using a microplate reader. This experiment was run in triplicate for each sample.

Detection of apoptosis by AnnexinV EGFP/PI double marking method

Apoptosis was detected by flow cytometric analysis. Briefly, the cells were washed and resuspended at a concentration of 5×10^5 cells/mL. Then, the cells were stained with 500 μl of Binding Buffer suspension cells, 5 μl of Annexin V-EGFP, and 5 μl of propidium iodide. After incubation at room temperature in the dark for 15 min, the cell apoptosis was analyzed on a FACSCalibur.

Analysis of cell cycle by flow cytometry

Transfected cells were harvested, fixed overnight with 75% ethanol at -20°C, washed three times with PBS, treated with RNase A (1 μg/ml), and then stained with propidium iodide. After propidium iodide staining, the cells were analyzed by flow cytometry, and cell proliferation index (PI) was calculated as below, PI (%) = (S+G2/M) ÷ (G1+S+G2/M) × 100%.

Reverse transcription PCR

The cycling protocol used was 5 min at 94°C, then 30 s at 94°C, 90 s at 59°C and 30 s at 72°C for 30 cycles and finally 7 min at 72°C. PCR was performed in a thermal cycler.

Reverse transcripts were used in the PCR reaction with the following primers.

The primer sequences were as follows: DEK: Forward, 5'-GTTGATTCTGGGGAGCCGAG-3'; Reverse, 5'-AGTGCCTGGCCTGTTGTAAA-3'. β-Actin: Forward, 5'-GCGTCGTCGACAACGGCTC-3'; Reverse, 5'-CAAACATGATCTGGGTCATCTTTC-3'.

RT-PCR result determination

Carrieing on the quantitative analysis of mRNA electrophoresis bands, to determine the gray value of hybrid bands; By using beta actin grey value in the band as a reference for correction all the relative expression of mRNA of samples were calculated separately. Calculation method is: Objective mRNA expression of relative amount = purpose gene mRNA stripe grey value/beta actin mRNA stripe grey value.

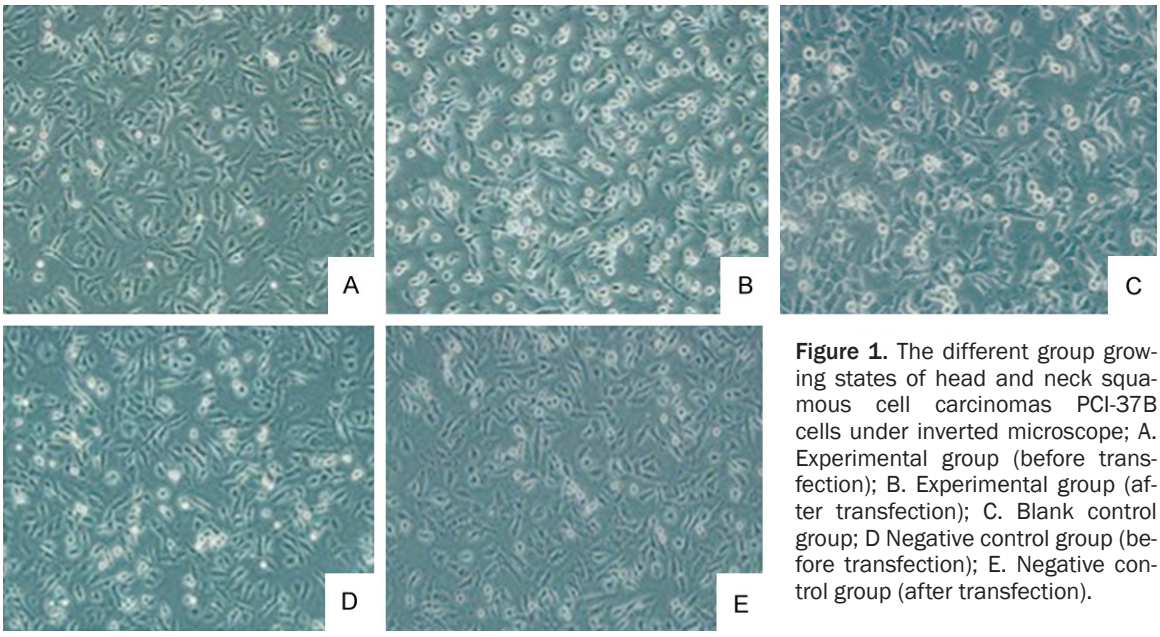

Figure 1. The different group growing states of head and neck squamous cell carcinomas PCI-37B cells under inverted microscope; A. Experimental group (before transfection); B. Experimental group (after transfection); C. Blank control group; D Negative control group (before transfection); E. Negative control group (after transfection).

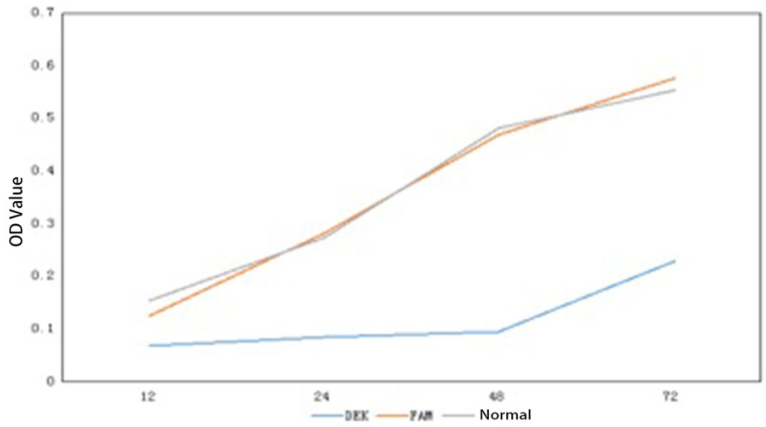

Figure 2. The inhibition of oral squamous carcinoma PCI-37B cell proliferation in different time points after DEK gene silencing.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 software (Chicago, IL, USA). Data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Student's t-test were used to analyze all experimental data. A *P*-value of <0.05 was considered statistically significant.

Result

Effect of DEK gene silencing on proliferation in OSCC CELL

The samples were observed under inverted microscope, after 24 hours transfection. In the experimental group part of the cells turn round other than shrunk after 48 hours and floating cells were increased; In contrast, in the blank control group and negative control group, most of the cells remain normal, and grew well with adherence (**Figure 1**). The result of by MTT assay showed that the OD value of experiment group is (0.094± 0.083), the negative control group and blank control group is (0.469±0.110) and (0.481±0.070), respectively. Compared with the experimental group, the difference had statistical significance (P<0.05). No difference was found in the negative group when compared with the blank group (P>0.05). Which indicated that the transfection of DEK siRNA could restrain proliferation of OSCC PCI-37 B, and the inhibition of cell proliferation was peaked at 48 hours after transfection and successful cultivation, the results are shown in (**Figure 2**).

Effect of DEK gene silencing on apoptosis in OSCC cells

At 48 hours after transfection. AnnexinV-EGFP/PI labeled method was used to detected cell apoptosis. The detection results were shown in

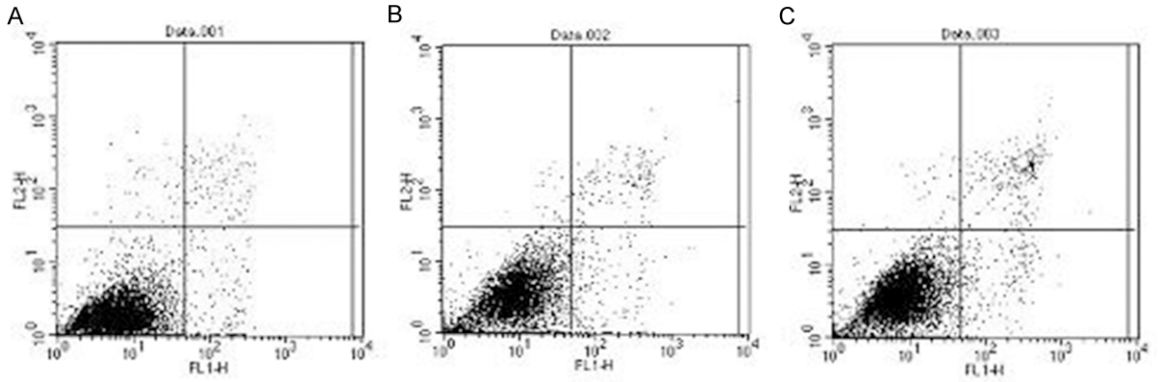

Figure 3. AnnexinV-EGFP/PI detected the different experimental apoptosis results; A. Blank control group; B. Negative control group; C. Experimental group.

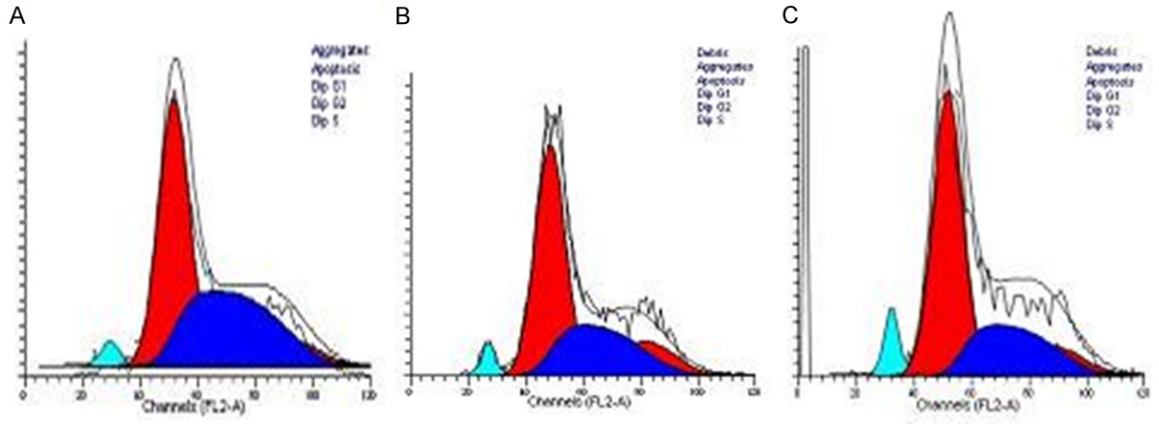

Figure 4. The analysis results of different experimental cell cycle; A. Blank control group cells; B. The negative control cells; C. The experimental group cells.

Table 1. The change of different group of cell cycle and cell proliferation index

Group	G_0/G_1	G2/M	S	PI (%)
Normal cells	44.54±2.37	15.81±1.99	34.06±5.23	52.67
FAM	48.68±1.37	12.78±3.24	40.21±1.46	52.07
DEK	52.93±0.60*	15.92±4.06	31.15±3.77	47.07*

Note: Compared with normal cells, *P<0.05.

Figure 3. The apoptosis rate of the experimental group was (5.72±0.33)% (P<0.05). While, when comparing negative control group with blank control group, the difference was not statistically significant. The results suggested that the inhibition of DEK gene expression could promote the apoptosis of OSCC cells.

Effect of DEK gene silencing on cell cycle in oral squamous cell carcinoma

The G0/G1 phase ratio of the blank control group and the negative control group was (44.54±2.37)% and (48.68±1.37)%, respectively. The experimental group was (52.93±0.60)%. Compared with the two control groups, the number of G0/G1cells in the experimental group was increased (P<0.05), Experimental cell proliferation index (47.07+0.59)% was significantly lower than that of blank control group (52.67+2.41)% and negative control group (52.07+2.63)%, (P<0.05) (**Figure 4** and **Table 1**). The results showed that the inhibition of DEK gene could interfere with cell cycle, for the proportion of cells in G0/G1 phase was increased significantly.

DEK mRNA expression

Under the condition of successful transfection specificity DEK-siRNA, the DEK mRNA was down-regulated in experimental group cells

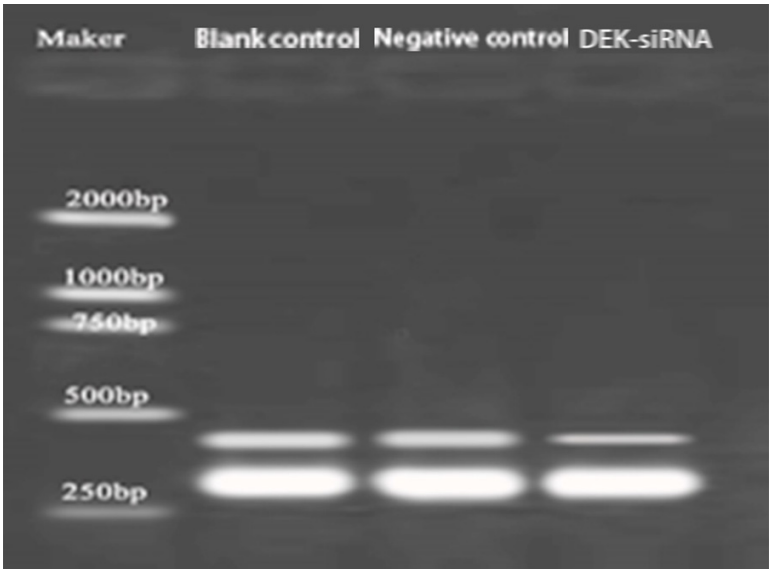

Figure 5. The detection of DEKmRNA gene experssion by PT-PCR.

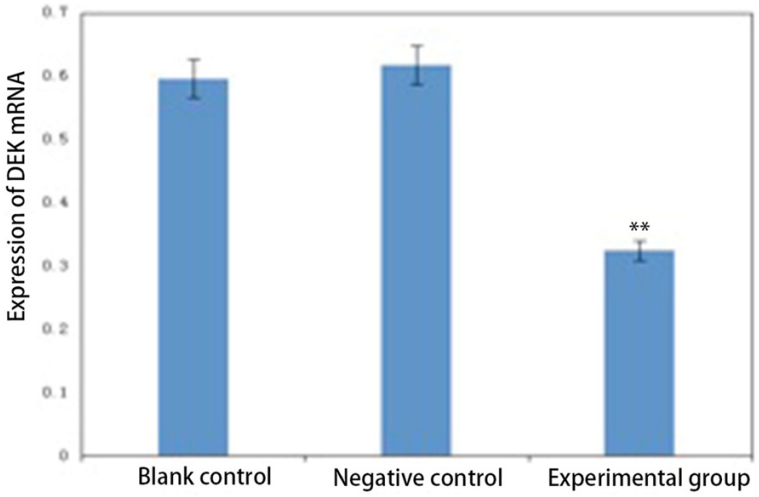

Figure 6. The DEK mRNA expression intensity of different group head and neck squamous cell carcinomas PCI -37 B cells.

after 48 hours, and the expression intensity was 0.325±0.015. The expression intensity of negative control group and the normal control group was 0.618±0.027 and 0.5964±0.031, respectively. The experimental group compared with the two groups, P<0.05 (**Figures 5**, **6**). This result showed that the target gene DEK siRNA can inhibit the expression of DEK mRNA gene in PCI-37B cells.

Discussion

The DEK protein, the topic of this essay, consists of 375 amino acids with four distinct stretches of acidic amino acids. It has a central SAP box DNA-binding domain and an additional caxboxy-terminal DNA-binding region in which are clustered by several phosphorylation sites [1, 6, 15, 19, 20]. The localization of DEK is throughout the cell cycle and it is always on chromatin and as a component of mitotic chromosomes. Its expression changes with the physiological situation of cells, which is higher in proliferating and lower in resting and terminally differentiated cells [21]. At present, the enterprise has been identified as a kind of original oncoprotein, which has been expressed in many human invasive tumors, such as retinoblastoma, hepatocellular carcinoma, colon cancer, bladder cancer, cervical cancer, malignant glioma, melanoma, and had an expression in gastric cancer. DEK can affect the proliferation, differentiation and apoptosis through binding to DNA and has an effect on cell apoptosis related factors [22, 23], it play an important role in the occurrence and metastasis of OSCC.

Our previous study revealed that DEK mRNA and DEK protein expression in OSCC and metastasis lymph node tissue were higher than tissue adjacent normal tissue, suggesting that the DEK proto-oncogene was closely related to the occurrence of OSCC. In this study, DEK - SiRNA was transferred into PCI -37 B cells, and we found that the proliferation of tumor cells was obvious inhibited with the decrease of DEK gene expression. When compared with the negative control group and blank control group, (P<0.05), which indicated that inhibition of DEK gene expression can effectively inhibit the proliferation of oral cancer cells, meanwhile, it found that the G0/G1 phase cells in the experimental group were more than

those in the control group, which indicated that the inhibition of DEK gene may be arrest cells in G0/G1 phase and then impacted proliferation and differentiation.

Previous studies had indicated that DEK had two-phase effects which promote or inhibit effect on cell apoptosis [22, 24, 25]. The result of this experiment showed that apoptosis rate of DEK gene was significantly higher than that of the negative control group and blank group, the differences were statistically significant, $P<0.05$. The inhibition of DEK gene can promote the apoptosis of OSCC, which may be related to the apoptosis related regulatory factors associated with DEK protein. But the specific mechanism have remained relative unexplored.

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Disclosure of conflict of interest

None.

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