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Original Research Article

CD164 induced the apoptosis of U87 cell by down-regulating PTEN/PI3K/AKT signal pathway in vitro

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To investigate CD164 induced the apoptosis of U87 cell by down-regulating PTEN/PI3K/AKT signal pathway in vitro. Human CD164 sequence was used for the design of shRNA targeting CD164, which was then introduced to lentivirus, followed by transfection into U87 cells. The CCK-8 was used to investigate whether silencing of CD164 exert anti-proliferative and pro-apoptotic effects on U87 cells. Western blot assay was used to detect the expression of PTEN, p-AKT and p53 in U87 cells. After transfection with CD164 shRNA in U87 cells, human glioma cell line U87 with down-regulation of CD164 showed significant inhibition of cell proliferation compared with shRNA NC (P<0.001). Furthermore, CCK 8 result showed that apoptosis rate induced by the CD164 shRNA transfection was markedly higher than that of control (P<0.001). The western blot result demonstrated that the silencing of CD164 increased the levels of PTEN and p53, whereas the level of p-AKT was decreased by transduction of CD164 shRNA. CD164 may induce the apoptosis of U87 cell by down-regulating PTEN/PI3K/AKT signal pathway in vitro, providing a novel ideal to improving the diagnosis and treatment of glioma patients.

Key words: CD164, U87, PTEN, apoptosis.

INTRODUCTION

Glioma is the most common malignant tumor of the nervous system. Nowadays many new therapies for glioma such as immunotherapy and gene therapy are constantly emerging, which are expected to further improve the prognosis of patients with glioma (Jiang et al., 2015). CD164 is a sialic acid mucin located on the No.6 chromosome (Hu et al., 2016).Studies have shown that CD164 played a role in regulating the growth, apoptosis and invasive behavior of solid tumors such as colon cancer, cervical cancer and medulloblastoma (Forde et al., 2007) ;(Shi et al., 2014). The research results showed that CD164 can be used as a molecular biological marker for acute lymphoblastic leukemia (Coustan-Smith et al., 2011).

Other researcher also found that inhibiting the activation of PTEN/PI3K/AKT pathway effectively inhibited the proliferation and metastasis of malignant tumor cells (Tang et al., 2012). However, there was no study on the correlation between CD164 and brain glioma yet, so this study is to investigate the effect of CD164 on the apoptosis of U87 cells in vitro and to analyze the possibility that CD164shRNA may induce the apoptosis of U87 cell by down-regulating the PTEN/PI3K/AKT pathway.

MATERIAL AND METHODS

Extraction and detection of mRNA

The steps of the extraction of mRNA were strictly in accordance with the procedure of miRNA extraction kit.CD164 upstream primer sequence was 5′-GCTTCGCCAATTTGGGGTTG-3′ and downstream primer sequence was 5′-TATCCATCATTGTCGAATGTGT-3′. The upstream primer sequence of Internal reference U6 was 5′-CCTCTTAGATCACTATGGCAG-3′. Its downstream primer sequence was 5′- GTTATTGAGCCATGGTTCGG-3′. According

to the instruction of manufacturer of PrimeScript™ RT-PCR kit, cDNA was produced through reverse transcription. mRNA was performed with a quantitative analysis as follows: 2min at 95°C, followed by 45-second reaction at 95°C and 60-second reaction at 60°C, repeated for 30 cycles, each sample was detected for 3 times, the relative expression difference multiples of CD164mRNA was calculated by the method of $2^{-\Delta\Delta Ct}$, the experiment was repeated for 3 times.

Reagent

Opti-MEM Medium, DMEM culture medium and fetal bovine serum were purchased from the Gibco Company. Trizol Reagent and Lipofectamine2000 were purchased from the Invitrogen Company. ShRNA NC and CD164 shRNA were purchased from GenePharma. MiRNA extraction kits, reverse transcription kits, RT-PCR kits and cell protein extraction kits were purchased from TaKaRa Joint-Stock Company in Japan. CCK-8 reagent kit was purchased from Nanjing Kaiji Biotechnology Development Limited Company while PTEN, p-AKT, p53 and β-actin antibodies were purchased from the Epitomics company.

Cell culture

Glioma cell line U87 cells was provided by Shanghai Institute of Cell Biology, which was cultured in DMEM medium in which was supplemented with 10% fetal bovine serum, penicillin (100U/μl) and streptomycin (100μg/ml). The cell was cultured in a cell culture box containing 5% CO_2 at 37 °C.

Construction of U87 cell infected by Lentiviral vector and screening

According to the CD164 shRNA effective target sequence provided in the literature, 3 interference sequences and negative control sequences were designed and constructed , and the most effective interference sequence was screened out. According to the instructions of the manufacturer of Lipofectamine2000, the lentiviral expression vector of the optimal interference sequence was transfected to the HEK-293T cell through Lipofectamine2000, the virus supernatant was collected and the virus titer was measured before infected with the SW480 cells, and after the cell was infected with the virus fluid for 12 h, the culture medium was replaced by one containing 10% fetal bovine serum, the fluorescence of green fluorescent protein was observed under fluorescence microscope, and the infection efficiency was detected by flow cytometry.

Infection of CD164 shRNA and shRNA NC to the U87 cell

The U87 cells in the logarithmic phase were inoculated into 24-pore plate, with 2×10^5 cells in each pore, 5μl of CD164shRNA with a concentration of 20μmol/l was mixed with 5μl of infection reagent Lipofeetamine 2000, and then added to the cells, CD164shRNA's infection final concentration was 100 nmol/L, and the culture plate was incubated in cell incubator for 48h before the related functional detections and Western blot were conducted. shRNA NC was infected in the same way as CD164 shRNA.

Detection of cell proliferation with the method of CCK-8

The U87 cells were seeded into 96-pore plate with 5×10^3 for each pore. The complete culture medium was added to each pore till the cells were fused for 100μl at 37 ℃ in a cell incubator containing 5% CO2, Each hole was added with CCK-8 solution (0.01 ml per pore) and reacted for 1 h, the absorbance value（A_{490}）at 490 nm wavelength was determined by the microplate reader, the experiment was repeated 5 times and an average value was taken.

Detection of the protein expression in cell by Western blot

The U87 cells were washed three times in the cold PBS solution and were re-suspended in the cell lysis buffer (100μl/pore). the cell shred was placed on the ice for a 30-min reaction , and then centrifuged for 20 min at 4°C and 12000xg , after the supernate was collected , BCA protein Assay Kit was used to carry out a quantitative analysis of protein concentration. The Protein sample (30μg) was transferred to the 0.45μm nitrocellulose membrane after undergoing a polyacrylamide gel electrophoresis with 10% lauryl sodium sulfate, and then incubated overnight with 5% skimmed milk powder at 4 °C , the membrane reacted with the primary antibody for 30min at room temperature at 4°C and then incubated with the secondary antibody for 1 h at room temperature. The optical density value of the maternal protein was corrected by the ratio of the gray value of the internal reference β-actin protein band.

Statistical analysis

Statistical analysis was carried out using SPSS 22.0 statistic software package. T-test and variance analysis were used to statistically analyze the sample data that has been acquired, and the statistical significance value was set as $P < 0.05$.

RESULTS

The expression of silent CD16 can induce the apoptosis of U87 cell

The infection in U87 cells was detected by western blot method after the CD164 shRNA and shRNA NC were respectively infected into U87 cell. The results showed that CD164 shRNA could significantly downregulate the expression of CD164 in U87 cells. The difference was statistically significant ($P < 0.001$), while the infection by shRNA NC had no significant effect. CCK-8 results showed that there was no statistically significant difference in cell absorbance as compared with control group 72 hours after being infected with shRNA NC. The difference was

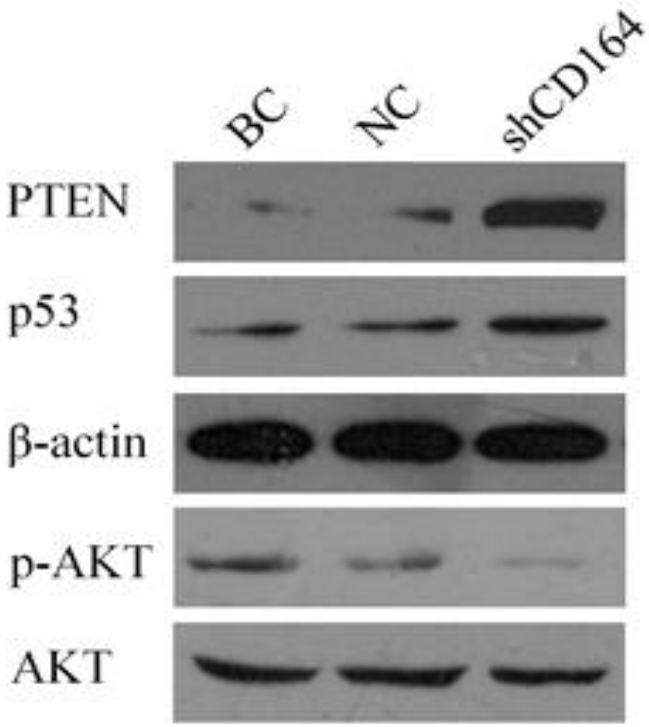

Figure 1: Western blot detection of the effect on expression of PTEN, p53 and p-AKT protein in U87 cell after infection by CD164shRNA

statistically significant ($P < 0.01$) when cell absorbance of U87 became lower than that of shRNA NC Group (43.8±7.1)% after being infected with CD164 shRNA.

Effect of expression of silent CD164 on the expression of PTEN, p53 and p-AKT in glioma cells in vitro

Western blot result showed that, compared with the negative control group, U87 cells could significantly upregulate the expression of PTEN and p53 protein in the U87 cell and at the same time inhibit the expression of phosphorylated AKT after being infected with CD164shRNA, while the shRNA NC had an insignificant effect on the expression of PTEN, p53 and p-AKT in U87 cell (Figure 1).

DISCUSSION

In this study, the expression of CD164 in glioma cells in vitro was silenced by using the RNA interference technique. The result of CCK-8 experiment showed that silencing the CD164 could inhibit the proliferation of glioma cells in vitro, and it was confirmed by the flow cytometry that the apoptosis of U87 cells could be induced significantly by down regulating CD164. It is indicated that the apoptosis of U87 cells can be induced after CD164 is downregulated, which may be the main mechanism of inhibiting the growth of glioma cells in vitro, but the specific intrinsic molecular mechanism is still not clear.

As a gene on chromosome 10q23.3, PTEN is also the second most susceptible gene found in the human tumor after p53 gene (Siddiqui et al., 2016). PTEN gene mutation or deficiency and abnormal protein expression can promote cell proliferation and inhibit cell apoptosis, the expression of PTEN in malignant tumors is negatively correlated with tumor TNM staging, and meantime, the low expression of PTEN is closely related to chemotherapy resistance of breast cancer (Song et al., 2016); (Wu et al., 2016). In this study, the author has observed that the expression of PTEN in cells can be induced by silencing the expression of CD164 in U87 cells, so it is suspected that the apoptosis of glioma cells induced by CD164shRNA may be related to PTEN pathway.

PTEN is regarded as an important regulatory protein in the signaling pathway of 3′-phosphatidylinositol 3-kinase (PI3K)/AKT. The absence or low expression of PTEN can inhibit the activity of PI3K kinase, causing the de-phosphorylation of PIP3, and leading to the amplification of expression of catalytic subunit of PIP3, which leads to phosphorylation of AKT, as a result, it plays an important role in many physiological activities like proliferation, apoptosis, protein synthesis and metastasis of malignant tumor cells, which is an important mechanism of PTEN in regulating tumor cell apoptosis. Previous studies have shown that inhibiting the activation of PTEN/PI3K/AKT pathway can effectively inhibit the proliferation and metastasis of malignant tumor cells (Smith and Briggs,

2016).

Furthermore, it is known that CD164shRNA can significantly upregulate the expression of p53 protein in glioma cells and inhibit the degree of phosphorylation of AKT, thereby it is indicated that CD164 may exert a certain influence on the apoptosis of glioma cells in vitro by regulating the expression of PTEN and its downstream protein, but it is currently not clear as to whether the pathway is the primary mechanism or the secondary mechanism, which still needs further study.

Conclusion

To sum up, this study shows that CD164 can negatively regulate the expression of PTEN in vitro, but it is still unclear as to whether CD164 has the same ability to regulate the glioma in vivo. It is also confirmed that CD164 is somewhat related to PTEN in glioma cells in vitro, it is uncertain as to whether this relation is influenced by other mechanisms. But this research still provides a new direction for clinical diagnosis and treatment of glioma, and it has a certain positive significance in revealing the pathogenesis and therapy of glioma.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

REFERENCES

Coustan-Smith E, Song G, Clark C, Key L, Liu P, Mehrpooya M, Stow P, Su X, Shurtleff S, Pui CH, Downing JR, Campana D (2011). New markers for minimal residual disease detection in acute lymphoblastic leukemia. Blood,117(23):6267-676. CrossRef

Forde S, Tye BJ, Newey SE, Roubelakis M, Smythe J, McGuckin CP, Pettengell R, Watt SM (2007). Endolyn (CD164) modulates the CXCL12-mediated migration of umbilical cord blood CD133+ cells. Blood,109(5):1825-18233. CrossRef

Hu J, Sun T, Wang H, Chen Z, Wang S, Yuan L, Liu T, Li HR, Wang P, Feng Y, Wang Q, McLendon RE, Friedman AH, Keir ST, Bigner DD, Rathmell J, Fu XD, Li QJ, Wang H, Wang XF (2016). MiR-215 Is Induced Post-transcriptionally via HIF-Drosha Complex and Mediates Glioma-Initiating Cell Adaptation to Hypoxia by Targeting KDM1B. Cancer Cell,,29(1):49-60. CrossRef

Jiang CM, Wang XH, Shu J, Yang WX, Fu P, Zhuang LL, Zhou GP (2015). Analysis of differentially expressed genes based on microarray data of glioma. Int J Clin Exp Med, 8(10):17321-17332.

Shi JA, Lu DL, Huang X, Tan W (2014). miR-219 inhibits the proliferation, migration and invasion of medulloblastoma cells by targeting CD164. Int J Mol Med, 34(1):237-243.CrossRef

Siddiqui S, Akhter N, Deo SV, Shukla NK, Husain SA (2016). A study on promoter methylation of PTEN in sporadic breast cancer patients from North India. Breast Cancer, 23(6):922-931. CrossRef

Smith IN, Briggs JM. (2016). Structural mutation analysis of PTEN and its genotype-phenotype correlations in endometriosis and cancer [J]. Proteins, 84(11):1625-1643. CrossRef

Song L, Liu S, Zhang L, Yao H, Gao F, Xu D, Li Q (2016). MiR-21 modulates radiosensitivity of cervical cancer through inhibiting autophagy via the PTEN/Akt/HIF-1α feedback loop and the Akt-mTOR signaling pathway. Tumour Biol, 37(9):12161-121. CrossRef

Tang J, Zhang L, She X, Zhou G, Yu F, Xiang J, Li G (2012). Inhibiting CD164 expression in colon cancer cell line HCT116 leads to reduced cancer cell proliferation, mobility, and metastasis in vitro and in vivo. Cancer Invest, 30(5):380-389.CrossRef

Wu YR, Qi HJ, Deng DF, Luo YY, Yang SL (2016). MicroRNA-21 promotes cell proliferation, migration, and resistance to apoptosis through PTEN/PI3K/AKT signaling pathway in esophageal cancer [J]. Tumour Biol, 37(9):12061-12070. CrossRef