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Construction of IL-18 Gene in Human Umbilical Cord Mesenchymal Stem Cells and Its Significance *

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ABSTRACT Objective: To construct IL-18 gene in human umbilical cord mesenchymal stem cells for exploring a means of tumor targeted gene therapy research. **Methods:** hUMSCs were isolated and cultured in vitro. The immunophenotype of hUMSCs was detected by flow eytometry. The lentivirus vector containing human IL-18 gene was constructed and transfected into hUMSCs. The IL-18 mRNA and protein expression level was detected by semi-quantitative RT-PCR and Western blot. **Results:** hUMSCs were isolated and cultured successfully. The FACS indicated that CD29,CD44 and CD105 were positive; However the CD34 and CD45 were negative. So it med the phenotype of hUMSCs. IL-18-hUMSCs was produced successfully. RT-PCR and Western blot showed that IL-18 gene was successfully transfected into hUMSCs and stable expressed. **Conclusion:** Lentivirus-IL-18 vector can be transfected and stable expressed in hUMSCs that it provided a new experimental method of tumor targeted gene therapy experimental study.

Key words: Interleukin-18; Human umbilical cord mesenchymal stem cells; Gene therapy

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Introduction

Gene therapy is an important measure to cure cancer. A lot of basic research had made many important achievements and showed good prospects. But how to achieve gene therapy of tumor, especially useing high efficiency, specificity and targetly gene therapy vectors, has been the focus of basic and clinical research. In recent years, it was found that bone marrow -derived mesenchymal stem cells (MSCs) of tumor tissue with chemotactic properties could be transfected with exogenous gene expression and could maintain its biological stability that became a highly effective targeted gene therapy tool[1,2]. Duing to the number of bone marrow-derived MSCs limites, its capacity of proliferation and differentiation decreases significantly with age that limites its wider application[3]. Relative to bone marrow-derived MSCs, human umbilical cord mesenchymal stem cells (hUMSCs) has no defects like that[4]. IL-18 is a newly gene that through a variety of ways to play the anti -tumor effect [5]. This study was to isolate hUMSCs from human umbilical cord and carry IL-18 gene transfected hUMSCs for preparing a stable expression hUMSCs IL-18 to provide an effective tool for experimental gene therapy in breast cancer.

1 Materials and methods

1.1 Materials and Reagents

Human umbilical cord was from health parturient with cesarean in obstetrics department of the Affiliated Hospital of Qingdao University Medical College following the agreements from the mother and her families; IL-18 gene carrying lentiviral and carrying a green fluorescent protein gene (GFP) were produced by Shanghai GenePharma Technology Co., Ltd; RT-PCR kit was purchased from Takara, Japan; IL-18 antibody of rabbit anti-human (ab68435) was purchased from American abcam company; Anti-GAPDH antibody and HRP -labeled IgG were purchased from Beijing Kang century biotechnology company; Mouse anti-human PE and CD29, CD34, CD44, CD45, CD105 of FITC -labeled monoclonal antibody were purchased from invitrogen company; RPMI1640, DMEM low glucose medium , trypsin and fetal bovine serum (FBS) were purchased from American HyClone company.

1.2 Methods

1.2.1 Isolation and identification of hUMSCs Full-term umbilical cord were collected Sterilely, and tissue was cut with phosphate buffered saline and washed repeatedly by using PBS to remove residual blood. The tissue was cut into small pieces as possible as 1-2 mm^3 and add a small amount of PBS from time to time to keep them moist. DMEM-LG medium was used to contain suspended tissue with 10% FBS and then inoculated into cell culture flasks. The flasks were inverted and placed in the incubator of 37 ℃ , saturated humidity and 5% CO_2. Umbilical cord mesenchymal stem cells was purified with repeated adherent. Then cell fusion was detected with an inverted microscope. When fusion was more than 80% , they were cultivated in 1:2 subculture with 0.25 % trypsin cells. A good growth between 3rd generation of umbilical cord mesenchymal stem cells was took, and washed 3 times with PBS. Mouse anti-human PE and CD29, CD34, CD44, CD45,

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CD105 of FITC -labeled monoclonal antibody and isotype control was added. Then it incubated in the dark and room temperature for 30min after washing with PBS with resuspended paraformaldehyde. It analysised with flow cytometry.

1.2.2 Lentivirus of IL-18 gene transfecting hUMSCs The hUMSCs cells were seeded in 96-well culture plate with 1×10^4/well, and each well of the culture medium volume was 100ul. It infected after 12-20h. According to the MOI (multiplicity of infection, MOI), the appropriate amount of virus was added. Viral load calculation method is(Cell number× MOI value / virus stock titer) × 10^3 = the amount of virus stock plus (ul). Plusing polybrene and changing the culture medium after 24 h. There is a total of three groups: the experimental group transfected with a lentivirus (IL-18-hUMSCs), transfected with blank control group lentivirus (NC-hUMSCs) and the control group did not transfected (hUMSCs). Transfected after 72 h, the expressions of each group was observed under fluorescence microscope. Analysising the efficiency of infection and selecting the optimal conditions.

1.2.3 Detecting the expression of IL-18-hUMSCs mRNA by RT-PCR Total cellular RNA was extracted by using Trizol reagent and transfected 5d later. Upstream primer of IL-18 gene was 5'-3'GAATAAAGATGGCTGCTGAACC,downstream primer was 5'-3'CCTGGGACACTTCTCTGAAA and amplified fragment was 439bp. GAPDH was used as internal reference and its amplified fragment was 146bp. With the reaction conditions of 95 ℃ 5min, 98 ℃ 10 s, 57 ℃ 30 s, 72 ℃ 30 s and 72 ℃ 10 min, and 35 cycles like that using RT-PCR. The products were taken after a 2% agarose gel electrophoresis to compare the control group and the blank control group.

1.2.4 Detecting the expression of IL-18-hUMSCs protein by Western blot Three groups of cells were collected with 1×10^6. After cell lysis centrifugation, the supernatant was used as the sample. Degenerating in 95 ℃ water bath for 10min and centrifuging, we stored supernatant was at -20 ℃. Using SDS-PAGE electrophoresis and after the end of it, the proteins was transferred to PVDF membranes. They were closed after adding IL-18 antibody of rabbit anti-human (abcam, 1:1000) and anti- GAPDH antibody whit 4 ℃ overnight. The first two days after adding the membrane was washed horseradish peroxidase (HRP) -conjugated secondary antibody (Beijing Kang century, 1:8000), incubated at room temperature for 1h and detected target protein by chemiluminescence.

1.3 Statistical analysis

The data were analyzed by using SPSS 17.0 statistical software. The databases were indicated by and the meaning between two independent samples is indicated by t text. Multiple samples are compared using analysis of variance that $P<0.05$ is considered statistically significant.

2 Results

2.1 Isolation and identification of hUMSCs

Separated hUMSCs were adherent growth like fusiform and decentralized form of proliferation of clonal colonies (Fig.1a). After lentiviral vector of IL-18 gene stablely transfecting hUMSCs, the cells had no significant changes in cell morphology and still were long spindle (Fig. 1b,c). Flow cytometry showed that CD29, CD44 and CD105 were (+), but CD34 and CD45 were (-). That met the hUMSCs phenotype(Fig.2).

Fig.1 The morphology of human umbilical cord mesenchymal stem cells a: 3rd generation of hUMSCs cells (normal light , × 100); b: infected hUMSCs cells 5d(normal light, × 200); c: perspective when infected the same hUMSCs cells 5d(fluorescence , × 200)

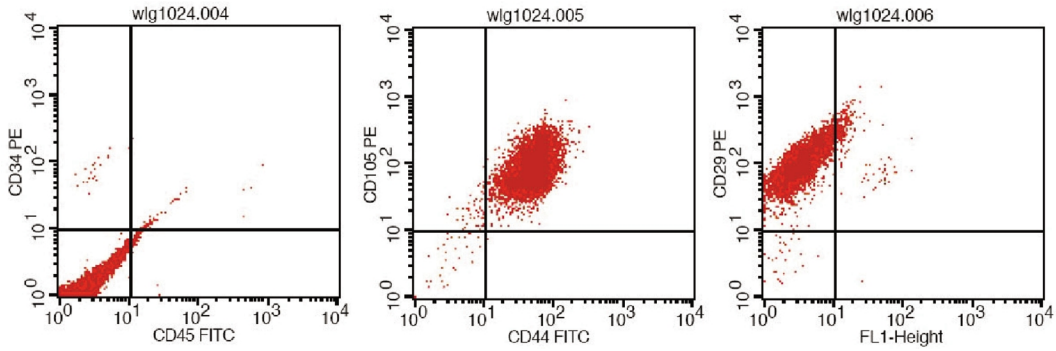


Fig.2 The phenotypic characteristic of human umbilical cord mesenchymal stem cell identified by Flow cytometry

2.2 Identification results of IL-18 transfecting hUMSCs

When hUMSCs was infected, the virus added to its cells with different MOI values. The results showed that when hUMSCs cells MOI were 70, 50, 30, 10I, infection group were 95 %, 80 %, 55 %, 25% (Fig.3). the value of MOI higher, the intensity of green fluorescence stronger. MOI were 50, 30, 10, and there was no significant cytotoxicity, but when hUMSCs MOI was 70 that had poor morphology. So this study determine that the best condition of infection MOI was 50.

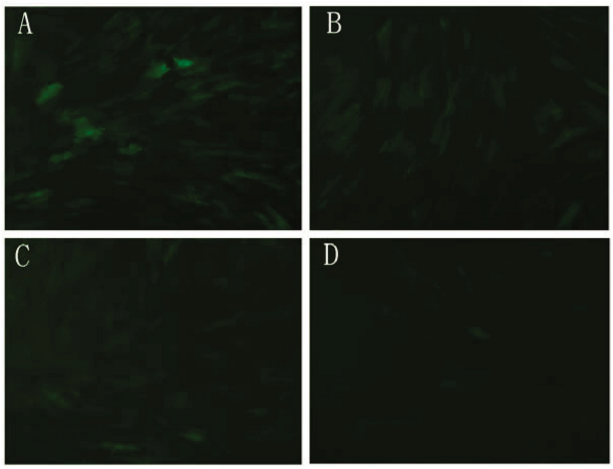


Fig.3 The images of infecting hUMSCs in different MOI values: A = 70, B = 50, C = 30, D = 10 (× 100)

2.3 The results of the expression of IL-18 mRNA

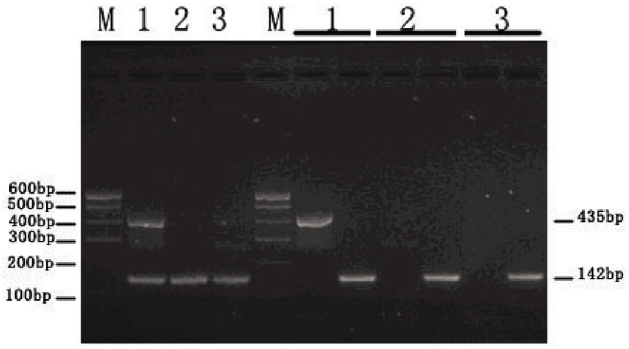
GAPDH (142bp) was an internal experimental group that IL-18-hUMSCs showed a specific band in 435bp and the relative expression of IL-18 mRNA was 1.38 ± 0.34. While there was no specific band in the control group (NC-hUMSCs) and the blank control group (hUMSCs), and the relative expression of IL-18 mRNA were 0.48 ± 0.18, 0.73 ± 0.29. The two control groups had no statistically significant (P=0.32). Comparing with that in the control groups, the expression of IL-18 mRNA in experimental group increased significantly. The differences were statistically significant(F=27.54, P=0.002)(Fig.4).

2.4 The expression of IL-18 protein

Transfected after 5d, there was the expression of IL-18 protein in the experimental group, the relative expression was 1.46 ± 0.42. While there was no expression of IL-18 in the control group (NC-hUMSCs) and the blank control group (hUMSCs), and the relative expression were 0.59 ± 0.25, 0.53 ± 0.36. The different of two control groups was not statistically significant (P=0.81). Comparing with that in the control groups, the relative expression of IL-18 protein in experimental group increased significantly. The differences were statistically significant (F=31.22, P=0.001)(Fig. 4).

3 Discussion

At present , the commonly used cancer gene therapy vectors



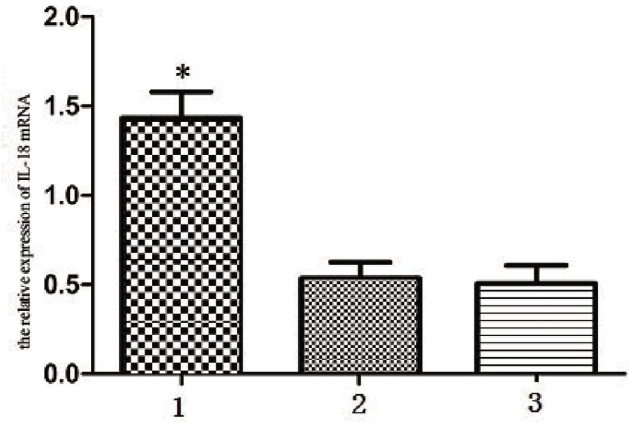
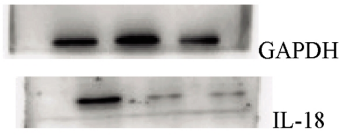


Fig.4 IL-18 mRNA expression and the relative expression of 1: IL-18-hUMSCs group 2: NC-hUMSCs group 3: hUMSCs group (a comparison with 2,3 , * P <0.05)



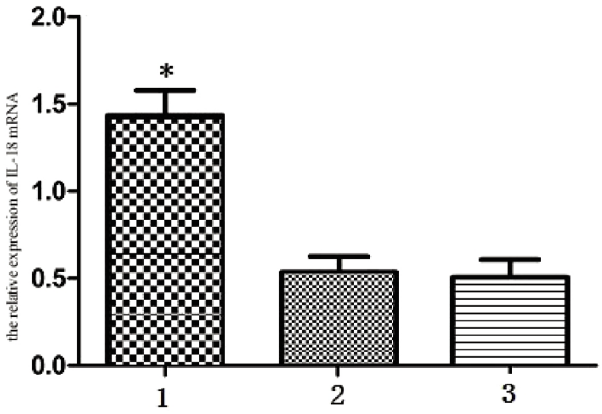


Fig.5 IL-18 protein expression and the relative expression of three groups 1: IL-18-hUMSCs group 2: NC-hUMSCs group 3: hUMSCs group (a comparison with 2,3 , * P <0.05)

are mainly plant viral vectors and non-viral body into two categories. Advantage of viral vectors is high transfection efficiency, but by the immunogenicity, cytotoxicity, limited of portable size and viral titer of exogenous genes easily raise other issues that plagued its research and application subject to certain restrictions[6,7]. Advantages of non-viral vectors are safe, simple to prepare and it can carry a great capacity of exogenous genes. But low transfec-

tion efficiency and shorter time of gene expression can also affect its application [8,9]. How to create a safe and effective therapeutic gene delivery systems become primary problem for the researchers.

MSCs is a pluripotent stem cell that has self-renewal, differentiation potential, proliferative ability and important biological characteristics of the tumor migration. It could be transfected with exogenous gene expression and could maintain its biological stability [10]. In particular, the ability to track MSCs chemotaxis can make it impossible to implement the migration to surgical resection and radiological tumor metastases that could not be found. Clearly expression of anti -tumor molecules by metastases can reduce the probability of tumor recurrence. Great values especially strongly of the treatment of metastatic tumors in cancer gene therapy field have broad application prospects [11-13]. In recent years, a large number of studies have showed that bone marrow-derived MSCs with age, significantly reduced the number of stem cells , proliferation and decreased in differentiation capacity, the risk of transplanted allogeneic immune has response and the patients have drawed some damage. So the amount of bone marrow extraction is restricted. The hUMSCs not only maintain the biological characteristics of MSCs, but also have more primitive, capable of proliferation, differentiation and low immunogenicity that generally do not cause an immune response. It isolated and cultured easy to operate, produce, store and transport. And cell damage caused by freezing small can be used repeatedly. The chance of infection and spread of latent viruses and pathogenic microorganisms are relatively lower, easily obtained. For maternal and newborn it has any damage and harm,and it has no morals and ethics limited etc[14-17]. IL-18 is a pleiotropic cytokine that can induce interferon γ (IFN-γ) production, enhance activity of natural killer (NK) cells and cytotoxic T lymphocyte (CTL), induce helper T cells (Th) and other biological functions. Through activation of T cells and macrophages it can induce apoptosis and inhibit tumor angiogenesis. Enhancing its cytotoxicity mediated by perforin or other factors have a synergistic anti- tumor or directly kill tumor cell, etc.It playes a strong anti-tumor effect[18-20].

This study successfully isolated hUMSCs from human umbilical cord, and carried IL-18 gene transfected lentivirus to hUMSCs. IL-18-hUMSCs build a stable IL-18 mRNA expression and protein, indicating that IL-18 is a hUMSCs effective means of delivery. IL-18-hUMSCs play MSCs targeting tumor characteristics and the anti-tumor effect of IL-18 that will became a new option of tumor targeting gene therapy.

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携带白介素 -18 基因的人脐带间充质干细胞的构建及其意义 *

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摘要 目的：构建携带白介素 -18（IL-18）基因人脐带间充质干细胞（hUMSCs），为肿瘤靶向性基因治疗研究提供一种工具。**方法：**体外分离培养 hUMSCs，流式细胞仪（FACS）检测 hUMSCs 的细胞免疫表型。应用基因重组技术将表达 IL-18 基因的慢病毒转染至 hUMSCs，利用 RT-PCR 及 Western blot 法检测 IL-18 的蛋白及 mRNA 表达水平。**结果：**成功在体外分离和培养了 hUMSCs，流式细胞仪检测结果显示 hUMSCs 表达 CD29、CD44 和 CD105，而不表达 CD34 和 CD45，符合 hUMSCs 的表型。成功构建携带 IL-18 基因的 hUMSCs，RT-PCR 及 Western blot 法检测结果提示 IL-18 基因转染至 hUMSCs 并能稳定表达。**结论：**构建携带 IL-18 基因的 hUMSCs 并稳定表达 IL-18，为肿瘤靶向性基因治疗实验性研究提供了一种新实验工具。

关键词：白介素 -18；人脐带间充质干细胞；基因治疗

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