Lethal Effect of T Cells Activated by Dendritic Cells with MUC1 Gene Transfection on BIU-87 Cells in vitro*

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Abstract Objective: To observe the lethal effect of T cells activated by dendritic cells (DCs) with MUC1 gene transfection (MUC1-DCs) on BIU-87 cells in vitro, and to evaluate the possibility of dendritic cells transfected by MUC1 gene as a vaccine for bladder cancer immunotherapy. Methods: MUC1 was successfully transfected into cultured human blood-derived dendritic cell with lipofectin. The transfection efficiency and immunocompetence were detected by flow cytometry and MTT colourmetry. T lymphocytes activated by MUC1-DCs were used to kill BIU-87 cell lines and normal bladder epithelium in vitro and the killing rate was evaluated by MTT colourmetry. Results: There was significant lethal effect on the BIU-87 cells and normal bladder epithelium with T cells activated by MUC1-transfected DCs, but the lethal effect on the BIU-87 cells significantly exceeded that on normal bladder epithelium (P<0.05). The lethal effect on BIU-87 cells with T cells activated by MUC1-transfected DCs significantly exceeded that with T cells activated by no-transfection DCs (P<0.01). Conclusion: T cells activated by MUC1-DCs could induce killing action to BIU-87 cell lines. MUC1 gene could be used as a target for bladder cancer immunotherapy.

Key words: dendritic cell; MUC1; transfection; bladder tumor

Dendritic cells (DCs) at present have been discovered to be of the highest functional specific duty antigen presenting cells. They can present antigen to T lymphocyte effectively and can induce specifically cytotoxic T lymphocyte (CTL) immunoreaction. CTL is a major effector cell to kill tumor cells in human body. So DCs has an important effect to induce the body to produce specific anti-tumor immunoreation.

MUC1 is a kind of glycoprotein at endothelial cells and expressed extensively and copiously on the cell surface. MUC1 core peptide-PDTRP epitope not only can be recognized by many kinds of MUC1 antibodies, but also can be recognized and killed by CTL. It had become an aggressive target by immunocyte. Moreover, MHC can’t confine it. So MUC1 is an ideal anti-tumor target molecule[2]. We use lipofectin to transfect human MUC1 full length cDNA gene to DCs. The in vitro killing experiment was performed with specific CTL induced by the post-transfection DCs-activated lymphocyte. By using upgraded MTT method, CTL lethal effects to normal bladder endothelial cells and cell line of transitional cell carcinoma of the bladder BIU-87 cells were evaluated and compared.

Materials and methods

Cell line and plasmid
Normal bladder epithelial cells and BIU-87 cells were bought from reservation center of Wuhan University, China. We serially subcultured them in our lab. Plasmid pVax-MUC1 including 32 tandem repeats was gifted by professor Taylor-Papadimitriou in Imperial Cancer Research Fund in UK.

Reagents
Lipofectamine 2000 was bought from Invitrogen company, rhGM-CSF, rhIL-4, rhTNF-a were bought from Cytolab company (Israel). FITC-CD83, FITC-CD86, FITC-MUC1 and PE-CD1a monoclonal antibodies were bought from eBioscience company (USA). MTT was bought from Atomic Energy Research Establishment of China. Complete substratum containing RPMI-1640 and 10% FCS was bought from Jinmei company (China). Ficoll lymphocyte isolation liquid was bought from Dakewe company.
Construction of MUC1 gene pcDNA3.1(+)−MUC1
MUC1 gene pcDNA3.1(+)−MUC1 was prepared in accordance with reference[2]. The gene sequencing of MUC1 was done by BoYa company (China). The result was consistent with human MUC1 gene sequences including foregone 13 amino acids signal peptide sequences.

Generous extraction and purity of plamid
By using plasmid macro-extracted kit, pcDNA3.1(+)−MUC1 was extracted and purified. By using ultraviolet spectrophotometer, the density of A260 and A280 was measured. The result of A260/A280 was 1.9522. The purified pcDNA3.1(+)−MUC1 was diluted into 1 g/L and stored at −20 °C.

In vitro culture of human blood-derived dendritic cells
Fresh blood 50 mL was taken from healthy volunteers. By gradient centrifugation density, peripheral blood mononuclear cells (PBMC) were separated and adhered about 2 h at 37 °C, then the supernatant was discarded and complete substratum RPMI-1640 containing rhGM-CSF 1000 U/mL and rhIL-4 500 U/mL added into the culture flask. The new culture fluid was changed after 3 days and surface marks of DCs including CD1a, CD83, CD86 were measured by flow cytometry 5 days later.

Transfer of pcDNA3.1(+)−MUC1 into DCs by lipofectin
Disposal of DCs: DCs which had been cultured 5 days were centrifuged at 800 r/min for 10 min, resuspended by lipofectin and adjusted to a density of 1×106/L. 400 µL cell liquid was put onto every well of 24-well plates and cultured under the condition of 37 °C and 5% CO2 overnight.

Lipofectin mixed with plasmid: 2.0 µL Lipofectamine 2000 and 0.8 µL pure pcDNA3.1(+)−MUC1 were added into 50 µL RPMI-1640 free of serum and antibiotic respectively. The mixture was mixed thoroughly and shaken slightly for 20 min.

Transfection of DCs: 100 µL mixture of DNA and lipofectin was added into the cell liquid in 24-well plates respectively. The plate was shaken slightly and put it into incubator at 37 °C, 5% CO2. The supernatant of mixture was discarded and the remaining was added with RPMI-1640 containing rhGM-CSF 1000 U/mL and rhIL-4 500 U/mL after 6 h and cultured for another 24 h. Untransfected DCs were taken as control group. The expression efficiency of MUC1 in post-transfection DCs was measured by flow cytometry.

Preparation of T lymphocyte
The PBMC were separated from peripheral blood of the same individual by Ficoll lymphocyte isolation liquid. The T cells were separated from PBMC through nylon hair pillar and were identified accredited by flow cytometry.

Results
Morphological observation and detection of DCs
The cultured cells which separated from PBMC were attached and aggregated as even dispersed small cell groups. Part of them were suspended after cultured for 5 days (Fig. 1, 2). The expression rate of CD1a, CD83, CD86 on the cell surface detected by flow cytometry was 63.7%, 42.3%, 76.8% respectively.