Antitumor Research of Murine Colon Carcinoma Cells Transduced with Mouse IL-21 Gene

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Abstracts **Objective** To investigate the antitumor responses of inhibiting the colon cells growth by interleukin(IL-21) gene tranferred into murine colon carcinoma cells. **Methods** We transduced murine colon carcinoma cells with the mouse IL-21 gene and select the high secretion cell strain, observe the effection of inhibiting the growth of colon tumor. **Results** Compared with the control cell group, the transfected Colon26/IL-21 cell strain could express IL-21 molecular and induce the large amounts of IFNγ secreted, which result in the Colon tumor evidently shrink. **Conclusions** IL-21 is a novel antitumor cytokine, may be selected as a waitful gene in gene therapy of colon carcinoma.

Key Words IL-21; Gene transfection; Colon carcinoma; IFNγ

In recent years, all kinds of anticancer therapy, such as radical operation, chemotherapy, radiotherapy, are widely used, but they have not made notable gains in five year survival rates. IL-21, a novel cytokine, has homologous structure to IL-2, IL-4, IL-15, and was relatively restricted expression in activated peripheral $CD4^+$ T cells. In this study, we shall transfect IL-21 gene into Murine colon carcinoma cells and exam its anticancer effects.

MATERIALS AND METHODS

Cells and mice

BALB/c mice were purchased from the experiment animal center of Hebei Medical University. Colon26 cells were provided by the cellular biology department of Hebei Medical University. Plasmid LXSN/IL-21, ecotropic ψ2 and amphotropic PA317 packaged cells were obtained from the pathologic research department Cancer Centre in Chiba, Japan. Liposomes Lipofectamine Regent, polybrene, G418 were provided from Sigma company.

Transduction of tumor cells

The retrovirus vector LXSN was used to harbor cloned IL-21 cDNA. The vector DNA with IL-21 cDNA was transfected into ecotropic ψ2 cells using lipofectin reagent and after the drug selection with G418(400μg/ml), cell-free supernatants of G418-resistant clones used as a retrovirus were incubated with amphotropic PA317 cells in the presence of 8μg/ml polybrene for infection. Among the G418-resistant PA317 cells, a clone that produced the largest amount of IL-21 mRNA was selected and culture supernatants were used for infecting Colon26 cells. G418 (400μg/ml)-resistant Colon26 cells were cloned and used for experiments.

Isolation of IL-21 cDNA

The reverse transcription-based polymerase chain reaction method was used to clone IL-21 cDNA. mRNA was extracted from G418-resistant Colon26 cells, and synthesized first-strand cDNA were amplified with two primers: 5′-ATT AAA GCT TCT GGT GGC ATG GAG AGG AC-3' and 5′-TAG GAT CCT GTG TTC TAG GAG AGA TGC TG-3′. Amplification was performed according to the manufacturer′s recommendation and it consisted of 35 cycles under the following conditions: 30s at 96℃ for denaturation, 30s at 56℃ for primer annealing, and 30s at 72℃ for primer extension. The length of amplified product was 434bp. We identified the expression level of IL-21 mRNA by ODIL-21/ODβ-actin.

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MTT assay

In the logarithmic growth period, Colon 26/IL-21 and Colon26 cells were digested by 0.04% EDTA and were inoculated in 96 cultured plate (1×10^5/ml). 37℃, 5% CO_2 cultured 6 days, then MTT (5mg/ml) was added to each hole. After 4 hours, we added DMSO (200μl/hole) was added to stop the reaction. Using enzyme-labelled device, OD data by 570nm wave-length and made a growth curve.

Flow cytometry

Parent or transduced cells were incubated with fluorescein-isothiocyanate-conjugated monoclonal anti-H-2Kd or anti-H-2Dd antibody (PharMingen, San Diego, CA) at room temperature for 20 min in the presence of 0.1% sodium azide. These cells were also reacted with fluorescein-isothiocyanate- conjugated rabbit anti-mouse immunoglobulin antibody. The stained cells were analyzed by FACScan(Becton Dickinson, Mountain View, CA) with CellQuest software(Becton Dickinson).

Production of IFN-γ

Spleen cells from the mice(1×10^7/ml) and those from the ice bearing either parent tumors or IL−21 producers were cultured. Cell-free supernatants collected after 48h were examined for the concentrations of IFN-γ. The data was statistized by SAS software.

Animal experiments

We chose 50 female mice and divided into two groups randomly. Colon 26/IL-21 and Colon26 cells were injected (1×10^7) into the back of mice. After 2, 4, 6, 8, 10, 12 days, tumor volume was individually calculated according to the formula 1/2 × length ×width ×height, and statistical analysis was performed by SAS software.

RESULTS

We gained the positive colon by G418 screening and found expression of IL-21 mRNA, while there was no expression in colon26 cell. (Fig. 1)

The transfected Colon26/IL-21 cell strain could normally proliferate in vitro, but its ability of proliferation had no obvious change in contrast to Colon26 cell strain. (Fig. 2)

The expression of MHC I molecular in Colon26/IL-21 cell increased at different degrees in contrast to that of Colon26 cell. (Fig.3)

The IFNγ secreted by Colon 26/IL-21 cell (179.67±11.32pg) was significantly higher than that of Colon26 cell (32.32±8.42pg) with ELISA. ($P<0.01$) (Fig.4)

After Colon26/IL-21 transfected, the growth rate in early stage was no difference compared with Colon26 cell. The Colon 26/IL-21 cell tumor regressed after the sixth day. The weigh of tumor was significantly different. ($P<0.01$) (Fig.5, 6)

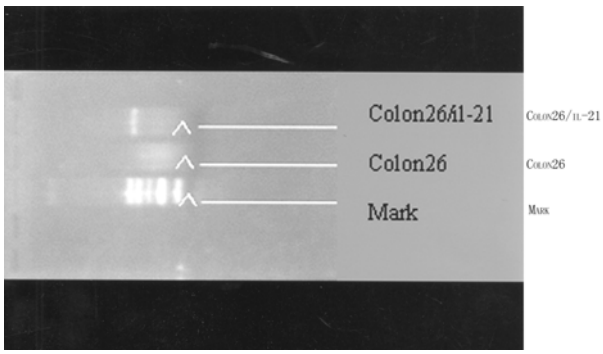

Fig.1 RT-PCR product of Colon26 and Colon26/IL-21celline

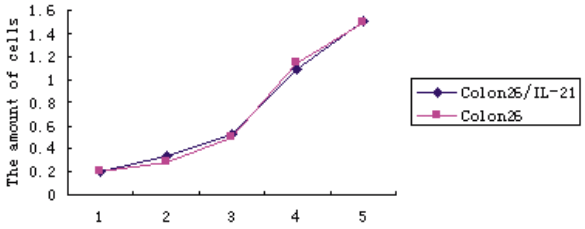

Fig. 2 The vigorous curve of colon 26/IL−21 and Colon26 cells

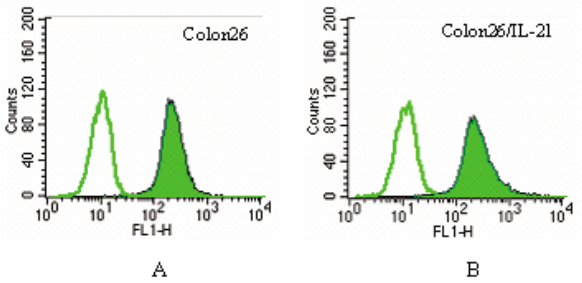

Fig.3 The changes of MHC class I moleculus of Colon26/IL-21 and Colon26 cells

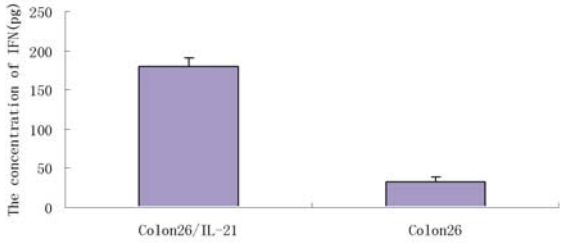

Fig.4 The IFNγ content secreted by spleen cell of experimental and control group

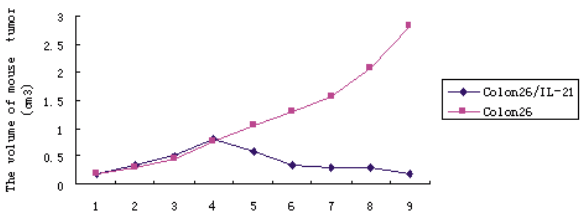

Fig.5 Chang of tumor volum after pad were transplanted with Colon26/IL-21 cells and Colon26 cells

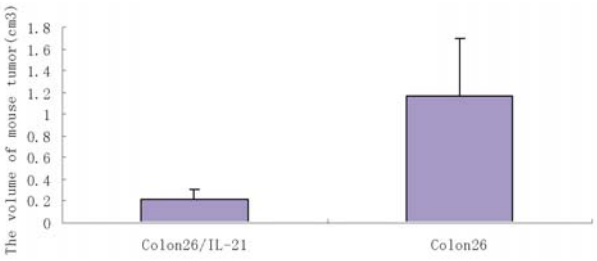

Fig.6 Tumor volume in the mice after transplanted Colon26/IL-21 cells and Colon26 cells

DISCUSSION

In recent years, more and more attention was paid to tumor immunity gene therapy. Zhang et al. applied vaccination IL-2 gene modified to treat gastric carcinoma, their results showed that the introduced cytokine gene could increase cell immune function and kill the tumor cells[1]. IL-21 could induce and activate the TH1 cell, which strengthened the cell immune function to produce lots of IFNγ and promoted both the proliferation of NK cell and the CTL effect in order to inhibit tumor growth. IL-21, as a novel cytokine, may produce antitumor effects, which attracted great interest of the clinical doctors.

IL-21 could increase the cell immune function in vivo. We observed the colon carcinoma cells with IL-21 transfected could decrease the tumor obviously, even make it regress completely. In normal conditions, IL-21 expressed on PMA or CD4+ T cell anti-CD3 and anti-CD28 activated. It was not only little secreted in normal condition, but also further less in the carcinoma patients[2]. We used reversed transcription virus as vector and insert IL-21 in the chromosome of colon carcinoma cell, and observed the cytokine could stably and constantly express in vivo, which not only stimulated the immune function, but also strengthened antigen-presenting effect. So it could inhibit the colon carcinoma cell growth or make the tumor regress[3]. During the therapy of colon carcinoma, we made the antitumor cell into vaccination gene modified to effectively decrease the postoperative recurrence and metastasis.

The transfected cell in the mouse inoculated Colon26/IL-21 constantly divided and proliferated to produce much IL-21. IL-21 combined with its receptor existing in the thymus, spleen, peripheral lymph cell and lymph node, which stimulated TH1 cell transfermation by activating JAK1, JAK3, STAT1 and STAT3 etc. The result was that the cell immune function was improved [4–6]. We found that the sensitized spleen cell of mouse secreted amount of IFNγ, which not only stimulated CD4+ T cell differentiation and strengthened CTL function of lymph nodes, but also increased NK cell proliferation and kill ability. Meanwhile, activated NK cell also increase secretion of IFNγ to kill the carcinoma cell[7–9]. In addition, from the flow cytometry, we compared Colon26/IL-21 cell with the control cell and found the expression of MHC I molecular increasing. It could stimulate the body to create the protective immune response. In vitro proliferation assay, it showed that the proliferation response between Colon26/IL-21 cell and wild Colon26 cell had no difference, that is to say, transfected cell had no neoplastic transformation. But we found there were 10% dead mice in Colon26/IL-21 during the assay, which was probably caused by over-degree immune response[10].

Above all, we observed IL-21 effect on colon carcinoma cell, the results showed that IL-21 could inhibit the tumor growth. Therefore, IL-21 may be a candidate gene to treat the colon carcinoma. However, Colon26/IL-21 cell strain successful establishment would be applied into the studies about action mechanism of IL-21, gene modified tumor vaccine and tumor gene therapy.

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