*Chinese Journal of Cazncer Research 9(4):281-284, 1997*

# EFFECTS OF IL-6/IL-2 FUSION GENE TRANSFECTION ON TUMOUR CELL BIOLOGICAL CHARACTERISTICS *IN VITRO* AND *IN VIVO*

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**In order to examine the feasibility of cytokine fusion gene transfer into tumor cells, a retroviral vector(pLXSN) for human interleukin 6/interleukin 2(IL6/IL2) fusion gene was constructed by using PCR and ligating the two genes with a synthesized oligonucleotides linker. The IL6/IL2 fused gene was introduced into B16 melanoma cell line mediated by retrovirus and the changes of adhesive and metastatic ability of fused gene-modified cells were detected. The B16-IL6/IL2 cells showed efficient expression of both cytokines and decreased binding affinity with extracellular matrix ( laminin and Matrigel), accompanying with decreased metastases potentials. These data suggest that the mechanism involving the decreased metastases may relate with the changes of biological characteristics and immune stimulating activity of gene-modified cells.**

**Key words : Gene transfer, Cytokine-fused-gene, Adhesion, Metastasis**

Transfection of cytokine genes into tumor cells allows delivery of cytokines at the site of tumor growth, where they might be expected to inhibit tumor growth and metastasis via effective induction of antitumor immunity. The studies of transfection IL-2 or IL-6 gene into tumor cells showed that the expression of IL-2 or IL-6 gene in a tumour cell line itself could locally alter the immunological micro-environment of the tumour cells so as to enhance the antigen presentation of tumour-specific antigens, and hence activate tumour-specific T cells.[1,2] Some works have demonstrated that the combination of the various cytokines in gene-transfer, could have synergistic effects in modulating tumor cell immunogenicity.[3,4] Since IL-6 can induce IL-2 and IL-2 receptor expression, the combination of IL-2 and IL-6 might be able to elicit a stronger response than with one cytokine alone. The aim of the present study was to investigate the effect in modulating tumor cell malignant phenotype by IL6/IL2 fused gene transduction.

Accepted Oct. 29, 1997
This study was supported by the National Postdoctoral Science Grant (中博基(1995)-7-8)

## MATERIALS AND METHODS

### Mice

Female C57BL/6 mice of 6-8 weeks old were purchased from the Military Academic Center for Experimental Animals.

### Cell Line and Tissue Culture

B16 melanoma cell line was grew in Dulbecco's modified Eagle's medium (Gibco), supplemented

with 10% heat-inactivated fetal calf serum (FCS), 100 units/ml of penicillin and 100μg/ml of streptomycin.

**Retroviral Construction and Cell Transfection**

The plasmids containing the complete coding region of human IL-2 and IL-6 were generous gifts from Dr. Nikki J. Holbrook and Dr. Toshio Hirano.

IL-6 cDNA were obtained by 30 cycles of PCR using primers modified to include EcoRI and NdeI sites at their ends. After the enzymatic cleavage, the inserts were ligated in the EcoRI and NdeI sites of the pBVIL2 plasmid which contain the oligonucleotides linker and IL-2 cDNA.[5] The fragment of IL6-linker-IL2 was obtained from the plasmid pBVIL6/IL2 by digestion with restriction enzymes EcoRI and BamHI and was inserted in the EcoRI and BamHI sites of the pLXSN retroviral vector (courtesy of Dr. YJ. Guo) to obtain the IL6/IL2SN vector. Retroviral vector constructs were converted to corresponding virus by using established procedures.[6] Supernatant with a high titer of virus (more than $10^5$ neo colony-forming units/ml) was used to infect melanoma cell lines. Clonal derivates of melanoma cells were isolated by G418 selection and expanded to cell lines, and IL-2 and IL-6 in the cell supernatant were measured by bioassay. Absence of replication-competent virus in the cytokine-producing melanoma cells was confirmed by NIH3T3 amplification assay.

**Cytokine Assays**

The IL-2 activity was determined by using IL-2-dependent cell line CTLL-2.[7] The IL-6 production was assessed by using IL-6-dependent cell line 7TD1 cells.

**Tumor Cell Adhesion Assay**

The cells detachment and adhesion assays were performed using the methods described by Sarkar[8] and Qian.[9] The extracellular matrix laminin(LN) and Matrigel™ were kindly provided by Prof. Zhou Rouli from Beijing Medical University.

**Spontaneous Metastasis**

Young (6-8 weeks ) female C57/BL6 mice were given subcutaneous injections into the right flank of either $10^5$ B16 melanoma cells, the mock-transfected cells, or the fusion gene-modified cells in a total volume of 0.2 ml. When the control group became moribund, the remaining animals were killed. The spontaneous metastasis rate in lungs was determined by histological sections and light microscopy.

**Experimental Metastasis**

Mice in each experimental group were inoculated intravenously with $5x10^5$ tumor cells. When the control group became moribund due to metastases, the remaining animals were killed. The lungs were assayed for metastatic load by weighting,and counting the number of metastatic nodules.

**Statistical Analysis**

Student's t-test was used for statistical analysis.

## RESULTS

**Cytokine Activity Assay**

Transfectants were selected with medium containing G418 while several clones of stable transfectants were established. Both IL-2 and IL-6 activity can be detected from the supernatant of IL6/IL2 fused gene transfectant. The activity of IL6/IL2 fusion protein is between hIL-2 at 200-600U/$10^6$cells/24h and hIL-6 at 40-60U/$10^6$cells/24h. Mock transfectant (B16-Neo) secreted neither IL-2 or IL-6. The growth rate of fusion gene-modified B16 cells in vitro was similar to that of parental B16 cells (date not shown).

**Effect of Fusion Gene-Transfer on Tumor Cell Adhesion**

We adopted two methods to detect the effect of IL6/IL2 fusion gene transduction on B16 cells adhesion potential. When compared with the parent B16 cells, the fusion gene-transferred B16 cells exhibited increased binding affinity to the plastic plates (Figure 1) and lower attachment to LN and Matrigel™ (Figure 2).

**Effect of Fusion Gene-Transfer on Tumor**

**Metastatic Potential**

Spontaneous metastasis was evaluated 30 days after subcutaneous implantation. B16-IL6/IL2 showed nonmetastasis. Experimental metastasis was evaluated 30d after intravenous inoculation. The fusion gene-transfectant also showed diminished metastatic loads of lungs (Table).

*Table. Effect of fusion gene transfection on tumor metastoses*

| Cell line | Spontaneous metastasis rate | *Experimental metastasi* | | |
|---|---|---|---|---|
| | | Colony count | Lung weight(mg) | *P* |
| B16 | 3/10 | 178 ± 22 | 954 ± 157 | |
| B16-Neo | 3/10 | 169 ± 28 | 926 ± 158 | |
| B16-IL6/IL2 | 0/10 | 44 ± 21 | 372 ± 79 | < 0.01 |

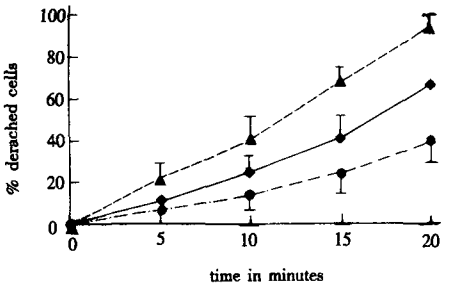


Fig.1. Assay the detachment of various groups of B16 cells from substratum with EDTA.

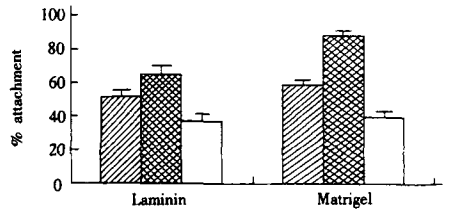


Fig. 2. Attachment of various groups of B16 cells to laminin and Matrigel.

## DISCUSSION

IL-2 is a growth factor for T-cells which enhances both non-specific immune responses such as NK and LAK cells as well as MHC-restricted cytotoxic T-cell responses.[10] IL-6 plays an important role in immune regulation via up-regulation of the humoral response. IL-6 is also a co-factor for T cell proliferation and differentiation and can induce IL-2 and IL-2 receptor expression.[11,12] The fusion protein secreted by the IL6/IL2 fusion gene-transfectant has both IL-2 and IL-6 activities, as the combination of IL-2 and IL-6 may have a good synergistic effect on inducing antitumor immune responses, the fusion gene transfection may have a better modification effect on tumor cells than single gene transfection. In the study of tumor biological characteristics , we do find that the tumor cells modified by the IL6/IL2 fusion gene showed a different changes on tumor adhesion characteristics as compared with single gene modified cells. The influence of fusion gene transfection on tumor cells growth, metastasis and tumor immunogenicity is also superior to that of single gene (unpublished date). There may be several mechanisms involving the decreased metastatic competence of fusion-gene-transfectant, including the decreased binding affinity to LN and Matrigel, and the fusion protein with both IL-2 and IL-6 activities may augment the cytotoxicity of NK, LAK , CTL and activate specific and non-specific antitumor immune responses. Other non-immune mechanisms may also be involved. IL-2 secreted by the cytokine gene modified tumor cells can induce the activation of the endothelium, and up-regulation of adhesion molecules on T cells, which results in enhanced migration of T cells to the tumour and elicited antitumor immune response.[10] IL-6 could induce protease inhibitors (TIMP) in fibroblasts and other connective tissue cells, thereby reducing the tumor's metastatic potential.[1] Furthermore, the cytokine gene transfection can directly increase the tumorigenicity and activate specific and non-specific antitumor immune responses, which may be the main mechanism to inhibit the tumor growth and metastatic potential.

## REFERENCES


1. Mullen CA, Coale MM, Levy AT, et al. Fibrosarcoma cells transduced with IL-6 gene exhibit reduced tumorigenicity, increased immunogenicity, and decreased metastatic potential. Cancer Res 1992; 52 : 6020.
2. Katsanis E, Orchard PJ, Bausero MA, et al. Interleukin-2 gene transfer into murine neuroblastoma decreases tumorigenicity and enhances systemic immunity causing regression of preestablished retroperitoneal tumors. J Immunother 1994; 15 : 81.
3. Cao X, Chen G, Zhang W, et al. Enhanced efficacy of IL-2 gene and IL-6 gene-transfected tumor cells in the treatment of established metastatic tumors. Gene Therapy 1996; 3 : 421.
4. Yannelli JR, Huatt C, Johnson S, et al. Characterization of human tumor cell lines transduced with the cDNA encoding either tumor necrosis factor α or interleukin-2. J Immunol Method 1993; 161:77.
5. Zhao CH, Tang PH, Wang JX, et al. Overexpression and characterization of recombinant human fusion protein IL-6/IL-2 (CH925). Stem Cells 1994; 12 : 339.
6. Gast G, Finstad CL, Guarini A, et al. Retroviral vector-mediated lymphokine gene transfer into human renal cancer cells. Cancer Res 1992; 52: 6229.
7. Tsai SCJ, Gansbacher B, Tait L, et al. Induction of antitumor immunity by interleukin-2 gene-transduced mouse mammary tumor cells versus transduced mammary stroal fibroblasts. JNCI 1993; 85 : 546.
8. Sarkar R, Das SK. Characteristics of retinoid-induced adhesion in a cultured human oral carcinoma cell line. Neoplasm 1992; 39: 87.
9. Qian YK. New practical methods of immunology. Beijing: Beijing Medical University & Peking Union Medical College Press 1994; pp. 43-45
10. Janssen RA, Mulder NH, The TH, et al. The immunobiological effects of interleukin-2 *in vivo*. Cancer Immunol Immunother 1994; 39 : 207.
11. Kishimoto T. The biology of interleukin-6. Blood 1989; 74 : 1-10
12. Gansman RD, Jacobs KA, Clark SC, et al. B-cell-stinulatory factor 2 functions as a second signal for interleukin 2 production by matur murine T cells. Proc Natl Acad Sci USA 1987; 84 : 7629.