Effect of targeted ovarian cancer therapy using amniotic fluid mesenchymal stem cells transfected with enhanced green fluorescent protein-human interleukin-2 in vivo

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Agenda Style

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Introduction
Introduction

Ovarian cancer is one of the most lethal types of gynecological malignancies. Chemotherapy is poor in recurrent patients. The aim of current research is to establish novel therapeutic methods.

Cell lines and animal models are valuable research tools. In our previous studies, the SKOV3 human ovarian carcinoma cell line was used.
introduction

IL-2

Function[4,5]

- stimulating the proliferation of T cells
- inducing cytotoxic T lymphocyte generation
- inducing the production of natural killer cells

Increase[6]

In cancer, including ovarian cancer, metastatic melanoma and renal cell carcinoma
Introduction

- when IL-2 is administered systemically at high doses have side effect:
  - malaise, fever, nausea, and vomiting\[7\]

- more severe reactions, hepatic dysfunction, increased capillary permeability and decreased systemic vascular resistance\[8\]

- therapeutic strategy that specifically delivers IL-2 to the tumor location may significantly reduce the required IL-2 dosage
introduction

Stem cells are becoming an important source of cells for cellular therapy\[^9\].

Mesenchymal stem cells are isolated from human second and third trimester AF and IL-2 and the green fluorescent protein (GFP) gene fused form a plasmid vector, transfected to MSC and intravenously injected at various doses into ovarian cancer nude mice.

Tumor formation, the expression hIL-2 were analyzed.

The aim of the study evaluate the migratory AF-MSCs into tumor cells in vivo, determine their function as delivery vehicles for anti-tumor molecules, such as IL-2.
Material method and Result
Isolation and culture of MSCs

- ethical approval.
- consent was obtained from each volunteer
- AF samples from 30 female volunteers
  - The samples centrifuged at 100 g for 5 min
- DMEM, FBS, Penestrep
- non-adhering cells were removed.
Markers of AF-MSCs analysed by RT-PCR

Total RNA extracted from MSCs using Tri Reagent, one step PCR

Primers oct 4

Primers B-actine

2) Tri reagent: guanidinium thiocyanate-phenol-chloroform
characterization Msc

- NTERA-2 cells line a pluripotent embryonic carcinoma as positive controls.
- MRC-5 cells line a human diploid fibroblasts lung tissue as negative controls.

1) Oct4 are transcription factors. It is critically involved in the self-renewal of undifferentiated stem cells.

2) Highly expressed in SC, embryonic carcinoma cell, embryonic germ cell.
EGFP gene transfection into MSCs

- 40-micro l Lipofectamine 20 h mixed with AF_MSC
- One week later, the stable transfectants were selected with G418
- Expression of EGFP in the cell monitored under UV microscope

G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic.
hIL-2 gene extraction

- Peripheral blood samples
- Lymphocyte separating medium centrifuged at 150 x g for 15 min
- First layer, plasma; second layer, lymphocytes and monocytes; third layer, lymphocyte
- Broken down using TRIzol. RNA extract. RT-PCR was performed to synthesize cDNA (462 bp)

Upstream primer: 5‘GGAATTCATGTACAGGATG3’
Downstream primer: 5‘GACTGAACTCAGCTGG3’
hIL-2 gene identification

Figure 7. Reverse transcription polymerase chain reaction analysis of hIL-2 gene expressed. Lanes: 1, water negative control; 2, marker; 3, hIL-2, hIL-2, human interleukin-2.
hIL-2 gene segment cloning and identification.

- Product was combined with a pMD18-T Simple Vector
- JM109 competent cells, heat shock
- Spread on (LB) agar plates with X-gal, digested with EcoRI and SalI, electrophoresis was performed

![Diagram of pMD18-T Vector](image.png)

Figure 8. Identification of the recombinant plasmid, pEGFP-hIL-2. Lane 1, 15,000-bp marker; lane 2, water negative control; lane 3, hIL-2; lane 4, pEGFP-hIL-2; lane 5, 2,000-bp marker. pEGFP-hIL-2, enhanced green fluorescent protein-human interleukin-2.
transfection of pMD-hIL-2 into AF-MSCs

- AF-MSCs were inoculated during the logarithmic growth phase
- pMD-hIL-2 was transfected into the AF-MSCs using Lipofectamine
- the cells were selected using 500 μg/ml G418
- hIL-2 detection kit, GFP gene expression detected fluorescence microscope
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Figure 10. Expression of the hIL-2 gene following transfection into amniotic fluid mesenchymal stem cells. OD, optical density; hIL-2, human interleukin-2.
Establishing an ovarian cancer animal

- SKOV3 ovarian cancer cells
- nu/nu-BALB/c nude mouse
- subcutaneously injected into the scapula region.
- 2 or 18 days before tumor cells inoculation
- tumor reached 1 cm in diameter
- AF-MSCs transiently transfected, pMD-hIL-2 were injected into the caudal vein of each ovarian cancer mouse
- Six weeks green fluorescence was apparent around the tumor or tissue
Establishing an ovarian cancer animal

Figure 12. GFP-labeled amniotic fluid mesenchymal stem cells surrounding the tumor mass. The GFP-specific signal as observed by (A) phase contrast microscopy (magnification, x50) and (B) fluorescence microscopy (magnification, x50). GFP, green fluorescent protein.
Ultrastructure examination of ovarian cancer cells.

Figure 14. Transmission electron microscopic observation of apoptosis in SKOV3 cells (magnification, x4,000). (A) Apoptotic SKOV3 cell displaying a swollen endoplasmic reticulum and (B) SKOV3 cell from the control group (magnification, x4,000).
Discussion
Ovarian cancer is a common type of malignant tumor.

It threatens the physical and mental health of females.

The majority of patients reached an advanced stage when they are diagnosed.

IL-2 administration stimulates T cell proliferation[10].

Physicians attempted to use IL-2 to cure patients suffering with metastasized ovarian cancer.

High concentrations of IL-2 are required.

MSCs systemic or local administration has been investigated in a variety of tumor animal models.
AF-MSCs may be considered as a powerful tool for gene therapy.

In the present study, AF-MSCs transduced with GFP were analyzed in a mouse ovarian cancer model to evaluate the migratory properties in vivo.

In the present study intravenously injected AF-MSCs, that stably express hIL-2,

are able to trace the subcutaneously transplanted ovarian tumor cells, and secrete IL-2 locally, resulting in the apoptosis of the tumor cells.


