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Study on interleukin-18 gene transfer into human breast cancer cells to prevent tumorigenicity*

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Abstract: To study the effect of interleukin-18 gene transfection on the tumorigenesis of breast cancer cell line Bacp37, human breast cancer cell line Bcap37 were transfected with Lipofectamine and selected by G418. The biological expression of rhIL-18 was tested by RT-PCR and ELISA method; nude mice were injected with Bcap37 cell with or without the hIL-18 gene. The hIL-18 cDNA was successfully integrated into Bcap37 cell; 126.3±4.5 pg hIL-18 secreted by one million transduced cells in 24 hours. Nude mice injected with IL-18 gene engineered Bcap37 cell had no tumor growth. These findings indicated that human breast cancer cells were successfully modified by the gene of IL-18 cytokine; the IL-18 gene engineered Bcap37 cells secreted hIL-18 and lost their tumorigenicity. The Bcap37 cells transduced with IL-18 gene may be used as breast cancer vaccine.

Key words: Interleukin-18, Breast cancer, Transfection, Tumorigenesis
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INTRODUCTION

Interleukin-18 (IL-18), originally described as interferon (IFN)-γ-inducing factor, is an 18.3-kDa proinflammatory cytokine produced by activated macrophages and dendritic cells, and plays an important role in the Th1 response, primarily based on its ability to induce IFN-γ production in T cells and NK cells. IL-18 induces proliferation of activated T cells, secretion of several cytokines, and participates in both innate and acquired immunity. The role of IL-18 in antitumor immunity was suggested in many studies. Gene transfer of cytokines is an important approach in tumor gene therapy. This study attempted to transfer the IL-18 gene into human breast cancer cell, and to investigate the effect of interleukin-18 gene transfection on the tumorigenesis of breast cancer cell.

MATERIALS AND METHODS

Human breast cancer cell line

The human breast cancer cell line Bcap37 was purchased from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences. Estrogen receptor status was negative. The cells were maintained in

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RPMI-1640 medium containing 10% fetal bovine serum, 100 units per ml penicillin and 100 μg per ml streptomycin. After transfection, the cells were cultured in RPMI-1640 containing 10% fetal bovine serum and 300 μg per ml geneticin (G418, Sigma, St. Louis, MO, USA).

Construction of pcDNA3.1-hIL-18

The targeting plasmid pcDNA3.1-hIL-18 was constructed by inserting a 554 bp IL-18 cDNA into BamHI and XhoI restriction sites of multiple cloning region on pcDNA3.1 plasmid, driven by a CMV promoter with a neomycin gene. The sequences were confirmed by the automated sequencer (ABI 373, Perkin-Elmer Applied Biosystems Division, CA, USA0).

Transfection

Before transfection, plate Bcap37 cells in 24-well plate, the cells will be 90%–95% confluent at the time of transfection. Dilute DNA in 50 μl of RPMI-1640 medium without serum. Mix gently. Mix Lipofectamine^{TM} 2000 gently before use, then dilute the appropriate amount in 50 μl of RPMI-1640 Medium (without serum). Mix gently and incubate for 5 minutes at room temperature. After the 5 minutes incubation, combine the diluted DNA with the diluted Lipofectamine^{TM} 2000. Mix gently and incubate for 20 minutes at room temperature to allow the DNA-Lipofectamine^{TM} 2000 complexes to form. Add the DNA-Lipofectamine^{TM} 2000 complexes to each well. Mix gently by rocking the plate back and forth. Incubate the cells at 37 °C in a CO_2 incubator for 24 hours for transgene expression, then replaced growth medium. Add selective medium (G418) the following day. G418-resistant colonies were isolated after 2 weeks of selection.

Detection of the IL-18 cytokine mRNA in transduced cells

The expression of IL-18 mRNA by Bcap37 cells before and after gene transfer were detected by reverse transcription polymerase chain reaction (RT-PCR). The RT-PCR process was as follows: mRNA was isolated by Tiozol, digested in Dnase I (GIBCO) for 1 hour to remove DNA, reverse transcripted into cDNA; after which reverse transcriptase was inactivated. DNA was amplified by PCR which consisted of 35 cycles including predenaturing at 94 °C for 4 min, denaturing for 1 min, annealing at 45 °C for 4 min and extending at 74 °C for 1 min. After 35 PCR cycles, it was reextended at 72 °C for 10 min. The RT-PCR product was detected by 3% agarose gel electrophoresis.

Human IL-18 Assay

Supernatant from 1×106 unmodified and IL-18 modified Bcap37 cells was collected after 24 hours. IL-18 activity was measured using IL-18 ELISA kit (Biosouce International Inc., Camarillo, CA).

In vivo animal studies

Parental, vector control cells, and transduced Bcap37 cells were removed by trypsin and washed twice with PBS, suspended at 1×107 cells/ml in HBSS and then injected sc into the dorsal flank of the nude mice. Tumor incidence and growth were recorded weekly for 4 weeks. Tumor volume was determined by multiplying the length, width, and thickness of the mass measured with a caliper.

Statistical analysis

The significance of differences in tumor takes was determined by the Sign test. A P value of <0.05 was considered significant.

RESULTS

In vitro characteristics of Bcap37 cell

An hIL-18 expression plasmid, pcDNA3.1-IL-18, was constructed by using a eukaryotic expression vector containing a neomycin-resistance gene. Bcap37 cells were transfected with pcDNA3.1-IL-18 or pcDNA3.1 by lipofectaine2000 and selected by G418, We have investigated the morphology of Bcap37-IL-18, Bcap37-pCDNA3.1 and Bcap37, there were no significantly difference.

Expression of IL-18 gene in transduced cells

The RT-PCR results on IL-18 gene transduced Bcap37 cell showed that: The PCR product of

Bcap37 cell colonies transfected with pCDNA3.1(+) -IL-18 was 570 bp. Nontransfected Bcap37 cell and the cell transfected with control vector did not express IL-18 mRNA (Fig.1).

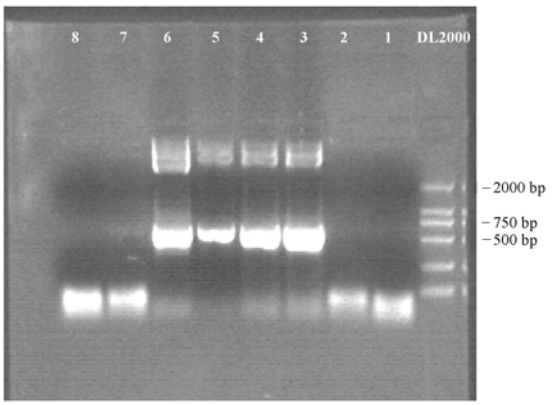

Fig.1 The results of RT-PCR

1,2: The Bcap37 cell colonies transfected with pCDNA3.1(+) vector; 3,4,5,6: The Bcap37 cell colonies transfected with pCDNA3.1(+)-IL-18; 7,8: The parental Bcap37 cell colonies

IL-18 ELISA assay

ELISA analysis showed that the IL-18 secretion rates of the 9 transfected Bcap37 cell lines ranged from 60.39 pg to 182.7 pg/24h. It was 126.3±4.5 pg hIL-18 in average secreted by one million transduced cells in 24 hours.

Tumorigenicity of transduced cells in nude mice

Parental Bcap37 cells have been shown to consistently form tumor in nude mice when injected into dorsal flank. Like parental Bcap37 cells, pcDNA3.1 vector control cells can also form tumor in nude mice. Nude mice injected with Bcap37-IL-18 did not form tumor. There was no significant difference in the rate of tumorigenicity between parental Bcap37 cells and pcDNA3.1 vector control cells (Table 1).

Table 1 Tumorigenicity of transduced cells in nude mice

Target cell		No. of animals developing tumors/total animals
Bcap37	10^7	5/5
Bcap37-pcDNA3.1	10^7	5/5
Bcap37-pcDNA3.1-IL-18	10^7	0/5

DISSCUSION

IL-18, formerly called interferon-λ (IFN-λ) inducing factor (IGIF), is recently discovered cytokine that plays an important role in the Th1 response, primarily by its ability to induce IFN-λ production in T cells and natural killer (NK) cells (Okamura *et al*., 1995a; Robinson *et al.*, 1997). IL-18 is produced by Kupffer cells, activated macrophages, keratinocytes, and intestinal epithelial cells (Pages *et al.*, 1999). Numerous investigations revealed importance of IL-18 as a Th1 cytokine, especially in cooperation with IL-12, in antitumor immunity (Okamura *et al.*, 1995a; Okamoto *et al.*, 1999). At the tumor site, locally decreased IFN production after decreased or abolished IL-18 production was correlated with an unfavorable outcome for patients with colon carcinoma (Dao *et al.*, 1998). IL-18 stimulates NK cells, T cells, B cells, and cells of the monocyte lineage to express IFN-γ at high levels (Okamura *et al.*, 1995b). Furthermore, IL-18 plays an important role in T-cell proliferation, CTL activation, and enhancement of NK cell activity primarily through the Fas-FasL mechanism (Hashimoto *et al.*, 1999). Systemic administration of rIL-18 is associated with significant in vivo antitumor effects that seem to be primarily mediated by enhanced NK activity (Ushio *et al.*, 1996; Osaki *et al.*, 1998). Recent studies, including one using IL-18-deficient mice, support the no- tion that IL-18 plays an important role in the development of cellar immunity (TH1 response) following administration of antigen (Micallef *et al.*, 1997a; Takeda *et al.*, 1998). This suggests that IL-18 has an important role in local antitumor immune responses.

Many studies revealed that tumor cells transduced with cytokine genes showed increased immunogenicity and decreased tumorigenicity, and that tumor vaccine prepared with tumor cells transfected with cytokine genes could induce potent antitumor cellular immunity (Robinson *et al.*, 1997). IL-18 exhibits antitumor activity in various tumor models. IL-18 induced the sequential activation of NK cells and CTL to protect syngeneic mice from transplantation with Meth A sarcoma (Micallef *et*

al., 1997b). Protective immunity was induced by the immunization with colon carcinoma cells genetically modified to express IL-18. Colon 26 cells transduced with the IL-18 gene (colon 26/IL-18) could not form subcutaneous tumors in immunocompetent mice, and the mice became resistant to rechallenge with wild-type colon 26 cells (Tasaki *et al.*, 2000).

In this study, human breast cancer cells were genetically modified by transferring the gene of IL-18 cytokine into the cells by Lipofectamine. An hIL-18 expression plasmid, pcDNA3.1-hIL-18, was constructed by using an eukaryotic expression vector containing a neomycin. The RT-PCR results showed: In IL-18 gene transfected Bcap37 cell, the PCR product was 516 bp. Nontransfected Bcap37 cell and the cell transfected with control vector did not express IL-18 mRNA. ELISA analysis showed that the IL-18 secretion rates ranged from 60.39 pg to 182.7 pg/24 h for the 9 transfected Bcap37 cell lines. In average, 126.3±4.5 pg hIL-18 was secreted by one million transduced cells in 24 hours. These results showed that the hIL-18 cDNA was successfully integrated into Bcap37 cell and could secret hIL-18 cytokine. Parental Bcap37 cells have been shown to consistently form tumor in nude mice. When injected into the dorsal flank, pcDNA 3.1 vector control cells, just like parental Bcap37 cells, can form tumor in nude mice. Nude mice injected with Bcap37-IL-18 did not form tumor. There was no significant difference in the rate of tumorigenicity between parental Bcap37 cells and pcDNA 3.1 vector control cells. The IL-18 gene engineered Bcap37 cell line lost tumorigenicity. The Bcap37 cells transduced with IL-18 gene may be used as breast cancer vaccine.

CONCLUSIONS

All of these findings indicated that human breast cancer cells were successfully modified by the gene of IL-18 cytokine. The IL-18 gene engineered Bcap37 cells can secreted hIL-18 and lose their tumorigenicity. The Bcap37 cells transduced with IL-18 gene may be used as breast cancer vaccine.

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