Construction of macrophage colony-stimulating factor receptor DNA vaccine

We amplified extracellular and transmembrane regions of macrophage colony-stimulating factor receptor (M-CSFR) and inserted them into pTARGET to construct a DNA vaccine, pCSFR. After immunizing BALB/c mice four times at one-week intervals, specific humoral and cellular immune responses were detected. The combination of pIL-18 enhanced CTL response induced by pCSFR. Furthermore, pCSFR had protective and therapeutics effect against tumor challenge in vivo.

DNA vaccination, which targets tumor-associated antigen (TAA), is a promising and rapidly developing strategy for tumor immunotherapy.[1] Scores of DNA vaccines against different TAAs have been developed, and six have been approved by the FDA for clinical trials.

Macrophage colony-stimulating factor receptor (M-CSFR), encoded by the c-fms proto-oncogene, is a receptor tyrosine kinase. Autocrine, intracrine and auto-juxtacrine mechanisms used by M-CSFR and macrophage colony-stimulating factor (M-CSF) were found to stimulate the growth of malignant cells while adhesion molecule-like roles were found in leukemia cells.[2-5] Point mutations and over-expression of both M-CSFR and M-CSF occur in solid tumors, Hodgkin's disease, and leukemias.[6-8] Treatment with anti-M-CSFR antibody or M-CSF soluble receptor inhibited the growth of leukemia and hepatoma cell lines bearing over-expressed M-CSF and M-CSFR.[4] So, M-CSFR is a potential target for tumor immunotherapy.

We used reverse transcription-polymerase chain reaction (RT-PCR) to amplify the 1682-bp cDNA fragment encoding extracellular and transmembrane regions of M-CSFR from the J6-1 leukemic cell line, and inserted it into pTARGET (Promega), a T-vector designed for mammalian expression, under the control of a CMV promoter to construct a M-CSFR DNA vaccine, designated as pCSFR. Sequence analysis revealed that the fragment was 99% homologous with the c-fms sequence (GenBank No. X03663) with three point mutations at amino acid level. To illustrate the successful construction of pCSFR further, COS7 cells were transiently transfected with pCSFR and the expression of M-CSFR fragment in mammalian cells was confirmed by Western blot analysis. To assess the immune response elicited by pCSFR in BALB/c mice, target cell lines that stably expressed the M-CSFR fragment (designated as SP2/0-CSFR) were obtained by transfecting SP2/0 cells with pCSFR followed by subcloning and G418 selection. The expression of M-CSFR fragment on SP2/0-CSFR subclones, one of which showing over 95% positive stain-

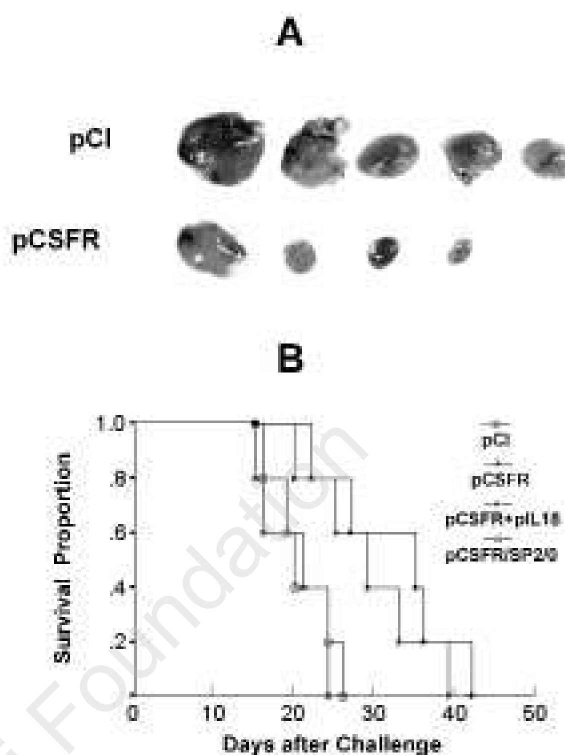

Figure 2. Protective and therapeutic effects of pCSFR against tumor challenge in mice. (A) Protective effects. Mice were immunized with pCI or pCSFR before SP2/0-CSFR challenge. Mice were killed 15 days later and tumor formation was analyzed. (B) Therapeutic effects. Tumor cells (SP2/0-CSFR for pCI, pCSFR and pCSFR+PIl18 groups; wild type SP2/0 for pCSFR/SP2/0 group) were injected intraperitoneally three days before plasmid inoculation on day 0 and day 10. The mice's survival was analyzed by Kaplan-Maier plots.

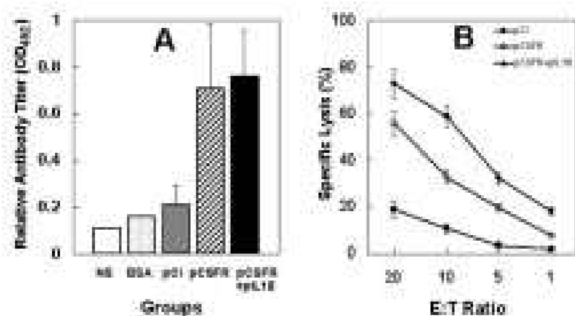

Figure 1. Immune responses in BALB/c mice induced by M-CSFR DNA vaccine. (A) The humoral immune response was evaluated by measuring, in triplicate, the M-CSFR specific antibodies in 1:50 dilution of serum samples from different groups indicated by ELISA one week after the last of four inoculations. Normal saline (NS) and BSA were controls for ELISA, while NS or 3% BSA was added instead of diluted serum samples. (B) Cellular immune response was evaluated by measuring, in triplicate, the M-CSFR specific CTL activities of mouse spleen cells from different groups indicated against SP2/0-CSFR cells by the LDH release assay one week after the last of four-inoculations according to the instructions provided by the CytoTox® 96 Non-Radioactive Cytotoxicity Assay Kit. The E:T ratio is the ratio of effector and target cells.

ing, were confirmed by immunocytochemistry and flow cytometry using monoclonal antibody against M-CSFR.

To assess whether pCSFR could induce specific immune response, endotoxin-free plasmids were purified following the instructions of the EndoFree Plasmid Mega Kit (Qiagen) and 100 µg of each purified plasmid was suspended in 100 µL sterile normal saline in aliquots for immunizing mice. Mice were injected with 100 µg[9] of either pCSFR or pCI (Promega), the blank parental plasmid of pTARGET, or a combination of pCSFR and pIL18, a mammalian interleukin-18 (IL-18) expression plasmid constructed in our laboratory by inserting the whole IL-18 coding region into pTARGET,[10] into the quadriceps muscle using an insulin syringe and needle. Booster injections with the same dosage were given in the opposite legs at one-week intervals for 3 weeks. One week after the final inoculation, specific antibodies against M-CSFR were detected by ELISA in 4 of 5 mice in the pCSFR and pCSFR+pIL18 groups but in none of the control group. The titer of M-CSFR specific antibodies was higher in samples from the pCSFR group ($p=0.002$) and pCSFR+pIL-18 group ($p=0.001$) than in those from the pCI group, while pIL-18 showed little effect ($p >0.5$ for pCSFR+pIL-18 compared with pCSFR) (Figure 1A). Lactate dehydrogenase release assay was done to determine M-CSFR specific spleen cell CTL activities against SP2/0-CSFR cells after immunization following the instructions of the CytoTox® 96 Non-Radioactive Cytotoxicity Assay Kit (Promega). This showed that the CTL activity in the pCSFR group was significantly higher than that in the pCI group while it was significantly higher in the pCSFR+pIL18 group than in the pCSFR group (Figure 1B).

To investigate the tumor protection effect of pCSFR in vivo, BALB/c mice were immunized with pCSFR or pCI three times at one-week intervals and then challenged with a subcutaneous administration of 1×10^6 SP2/0-CSFR on the fourth week. Mice were killed 15 days thereafter and tumor formation was analyzed: the average tumor weight in the pCSFR group was much lower than that in the pCI group (0.372±0.27g v.s. 1.498±0.92g, $p=0.024$) though 1 of 5 mice was protected from tumor. (Figure 2A). Tumor therapy experiments were done when 2×10^5 tumor cells were injected intraperitoneally, three days before plasmid inoculation on day 0 and day 10 at the dose of 100 µg each. The results showed that pCSFR markedly prolonged the survival of injected mice SP2/0-CSFR ($p=0.014$) but not the wild type SP2/0 ($p=0.652$) as compared with the pCI innoculation group by log-rank test (Figure 2B).

Our results suggested that M-CSFR DNA vaccine could induce specific humoral and cellular immune responses and have protective and therapeutic effects.

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