Chinese Journal of Cancer Research 9(3):192-194,1997.

THE EFFECT OF TGFβ1 ON MURINE TUMOR GROWTH FOLLOWING DIRECT INTRATUMORAL INJECTION OF PLASMID DNA*

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In order to investigate TGFβ1 gene expression and its effect on murine tumor growth following direct intratumoral injection of naked plasmid DNA encoding human TGFβ1, LM_3 murine lung adenocarcinoma cells were inoculated subcutaneously to T_{739} mice and grew to tumor nodules in 2 weeks. Multiple direct intratumoral injection of plasmid DNA, PMAMneo- TGFβ1, were given and compared with saline or vector plasmid administration groups. The growth of tumor was observed till the 8th week when the mice were killed for Northern blot analysis and histopathological study of tumoral tissue. The results showed that the growth of tumor was boosted in the TGFβ1 gene treated mice as compared with the control groups, whereas there was no significant difference in the metastatic behavior. Northern blot showed efficient expression of TGFβ1 mRNA in the treated group. The present study indicated that TGFβ1 may stimulate tumor growth *in vivo* through certain mechanisms. And direct intra-tumoral injection of nude plasmid DNA may be a promising gene transfer strategy *in vivo*.

Key word: Neoplasms therapy, Gene therapy, Gene transfer, TGFβ1.

The past decade has seen a great increase in laboratory studies and clinical trials in human gene therapy. In investigation of gene therapy, the strategies for gene transfer can be generally grouped into two categories: *ex vivo* and *in vivo*. To date, *ex vivo* approaches of transfecting cells from individual patients for gene therapy have been successful and account for the dominant reports in this field. However, there are some limitations to the techniques. These include the need to culture cells *in vitro* from each patient to avoid allogeneic tissue rejection, the tendency of cells to undergo phenotypic alteration in culture, the possibility of outgrowth of aberrant transformed cells, and the requirement of expertise to prepare cultured cells. Additionally, many *ex vivo* gene therapy protocols require transduction with an integrating retroviral vector prior to reintroduction of the cells, thereby raising safety concerns. To overcome these limitations, researchers have done a great deal in their investigation of direct *in vivo* gene transfer strategy and encouraging results have been reported.[1,2]

In our previous study, PLA-801D cells, a human pulmonary giant cell carcinoma cell line, were transfected *in vitro* with an expression vector containing a full length human TGFβ1 gene. The transfected cells showed growth inhibition both in the conditioned media and in athymic nude mice which were immune dificient.[3] Based on the results, the present study further introduced TGFβ1 gene to the

Accepted July 26, 1997
* This work was supported by the National Natural Science Foundation of China (No. 39370761).

tumors borne in the mice with normal immune response by direct intratumoral injection of naked plasmid DNA encoding human TGFβ1. The aim of this study was to investigate the efficiency of TGFβ1 gene expression following direct intratumoral adminis-tration of naked plasmid DNA and its effect on tumor growth in mice with normal immune response.

MATERIALS AND METHODS

Cell Line, Animals and Plasmid DNA

LM_3 cell line, murine lung adenocarcinoma cells with high metastatic potential, was established in our laboratory. T_{739} mice, 6–8 weeks old, male, were purchased from the Animal Center of Cancer Institute, Chinese Academy of Medical sciences. Plasmid DNA, PMAMneo-TGFβ1 encoding a full length human TGFβ1, was kindly donated by Professor Zhang HQ of Beijing Medical Research Institute. The preparation and structure of the plasmid DNA was published elsewhere.[4]

Reagents

RPMI-1640 medium and random primers DNA labeling kit was purchase from Promega Co. (Beijing). ^{32}P-α-CTP was otained from Ya Hui Company. A cDNA encoding 350 bp TGFβ1 fragment was used to prepare the probe for Northern analysis.[3]

In Vitro Molecular Manipulation

Extraction and quantitation of plasmid DNA, the digestion, extraction and labeling of the probe, the isolation of RNA and Northern analysis were performed by the methods described by Sambrook et al.[5]

In Vivo Gene Administration

T_{739} mice were randomly divided into 3 groups. Each group had 7 mice. LM_3 cells were cultured in RPMI-1640 medium and harvested according to standard procedure. 5×10^5 cells were inoculated subcutaneously into the back region of the mice. Tumor nodules were formed in all the mice in 2 weeks. Expression plasmid DNA PMAMneo-TGFβ1 (50 μg/50μl) was injected into the tumor nodules while the mice in 2 control groups were injected with PMAMneo vector DNA (50μg/50μl) or saline (50 μl) only. The growth of tumor was observed. Subsequent administrations were given at the 4th and 7th week (100μg/100μl). The mice were killed at the 8th week. Fresh tissues from the tumors were taken for RNA extraction. Tissues from the lung, lymph nodes, liver and tumor were also taken for pathohistological analysis.

RESULTS

Northern Blot Analysis

RNA was isolated from fresh tumor tissues. Northern blot revealed the presence of an intensive hybridization signal in each sample injected with plasmid DNA encoding TGFβ1, while the hybridization signal was barely detectable in the samples of 2 control groups. The results indicated that TGFβ1 gene achieved efficient expression following direct intratumoral injection of naked plasmid DNA.

Effect on Tumor Growth

Tumor nodules were formed subcutaneously in all the mice in 2 weeks post inoculation. Tumor growth was observed and tumor volume was determined from the formula $V=ab^2/2$, where "a" was the longest diameter and "b" was the shortest diameter of the tumor. Tumor growth curves were drawn (Figure 1) which showed that the increase of tumor volume in TGFβ1 gene injected group was faster than in the control groups. Pathohistological analysis of the tumor tissues revealed that in TGFβ1 gene injected group, interstitial CT tissues increased while infiltration of inflammatory leukocytes and lymphocytes decreased compared with the control groups. Metastasis to lung, lymph nodes, liver or spleen was observed in both gene treated group and control groups. No significant difference was detected.

DISCUSSION

In 1985, Roberts et al. presented evidence that the action of TGFβ1 on cells was bifunctional. And the bifunctional response of the cells to TGFβ1 could

not be ascribed to differences between nonneoplastic and neoplastic cell types. Rather, it could act either synergistically or antagonistically with other growth factors or conditions.[6] In our previous study, the growth of tumor cells transfected with TGFβ1 gene was inhibited both in conditioned media and in nude mice. However, in the present investigation, the tumor growth in mice with normal immune response after direct intratumoral administration of expression vector containing TGFβ1 gene was boosted, which was contrary to the previous findings. Although further and more systematic experiments are needed to confirm the results and to interpret its mechanisms, a possible explanation can be given so far is that, as Roberts stated, TGFβ1 might indirectly stimulate tumor growth by means of inhibiting immune response or immunological surveillance or by supporting tumoral matrix growth through autocrine or paracrine mechanisms.

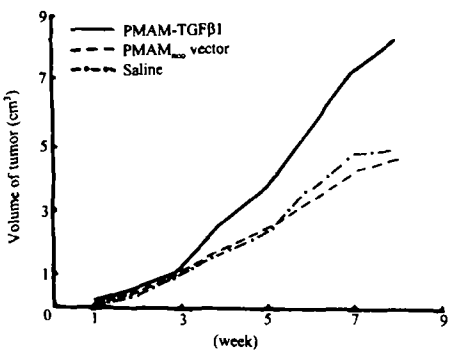

Fig. 1. Growth curve of the tumors

In recent years, there has been increasing interest in methods for gene delivery that do not require viral vectors or cell transplantation. Wolff et al. first reported in 1990 that RNA and DNA expression vectors containing report genes were injected into mouse skeletal muscle *in vivo* and protein expression was readily detected in all cases.[2] Subsequently, successful animal experiments of direct injection of genes to organs or tissues such as the liver, lung, thyroid, tumor masses, skin or endothelium have been reported.[1] Moreover, direct injection approach was also used in clinical human trials. Zaugg et al. injected directly plasmid DNA coding for HLA-B7 to tumor tissues in 2 patients with advanced skin cancer, who had both failed to respond to any other treatment, and great than 50 percent of the cancer was removed.[7] All these reports profiles the promising prospect of *in vivo* gene delivery approaches in gene therapy. In this paper, the results achieved may be preliminary and more systematic studies are needed. However, the fact that the directly intratumoral injected gene achieved sufficient expression might be enough to encourage us to continue our research.

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