

Gene therapy in prostate cancer: past, present and future

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1. ABSTRACT

The absence of effective therapies for hormone refractory prostate cancer establishes the need to develop novel therapeutic modality, such as a gene therapy, that can be applied either separately or in conjunction with current treatment modalities for the treatment of advanced prostate cancer. About 80 protocols for prostate cancer gene therapy have been practiced since 1994. The gene therapy modality is ideal for the treatment of prostate cancer. The disease progress can be precisely monitored by serum-PSA level, the local access is easy by ultra-sound guidance, and prostate as an accessory organ is highly immunogenic. Consequently, the number of prostate cancer gene therapy trials is increasing now. In this paper, we review the previous clinical trials of prostate cancer gene therapy in the chronologic order, and predict the future prospects.

2. BACKGROUND OF PROSTATE CANCER GENE THERAPY

More than 15 years have passed since the first gene therapy clinical trial for patients with ADA (adenosine deaminase) deficiency in 1990 (1), however, gene therapy has not reached yet the outcome initially expected. Furthermore, although over 6,000 patients have received gene therapy so far worldwide, there is actually no commercially approved drug for gene therapy except for the drug based on p53 gene, which was launched in October 2003 in the Chinese market (2). However, technologic innovations in the basic research field are remarkable, and it is sufficiently expected that these techniques should be adopted in clinical settings with certain their therapeutic effects. In U.S., approximately 800 gene therapy clinical protocols have been practiced so far,

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Table 1. Autologous vaccine vs allogenic vaccine

	Autologous Vaccine	Allogenic Vaccine
Advantages	Matching-Human Leukocyte Antigens/ Major Histocompatible Complex	Large-scale production, Ready to use
Disadvantages	Need patient's tumor, Difficulty of primary cell culture, Take long time to start the vaccination	Mismatching-Human Leukocyte Antigens/Major Histocompatible Complex

Table 2. Principal features of the vector system

Vector	Retroviral vector	Adenoviral vector	Adeno-associate viral vector	Naked DNA vector
Advantage	Long term gene expression	Concentrate high titer	Wide target cell range	Low cost, Non-toxic
Disadvantage	Oncogenicity	Transient expression	Limited insert size	Poor efficiency
Gene integration	Yes	No	Site-specific	No
Cell division requirement	Yes	No	No	No
Immunogenicity	No	Yes	No	No

and approximately 80 protocols for prostate cancer have been practiced since 1994. The first reason why the clinical trials in prostate cancer gene therapy are so popular in U.S. is that prostate cancer rank first in the cancer death in the U.S. males and that effective therapies for the patients with hormone-refractory prostate cancer has not been established (3). As additional reasons, the disease progress can be precisely monitored by serum-PSA (Prostate Specific Antigen) level, resistance to conventional therapies such as radiation-therapy and hormone-therapy can be easily detected even in the cases that cancer recurrence is barely confined, the local access is easy by ultra-sound guidance or the like. Consequently, the history of prostate cancer gene therapy may be the epitome of the history of general cancer gene therapy. This paper will present the progress and future vision of cancer gene therapy by outlining previous protocols for prostate cancer gene therapy, which are registered in the U.S. NIH (National Institute of Health) in the chronologic order.

3. FROM 1994 TO 1999

3.1. Immuno-gene therapy

The protocol for prostate cancer gene therapy which was approved by the NIH Recombinant DNA Advisory Committee (RAC) for the first time in 1994 was immuno-gene therapy using autologous GM-CSF (granulocyte-macrophage colony stimulating factor) gene-transduced tumor cells (4). In the immuno-gene therapy, the autologous GM-CSF-transduced tumor cells which were prepared by *in vitro* culturing the prostate cancer cells collected from the patient and subsequently introducing the GM-CSF gene by retrovirus were subcutaneously inoculated to the patients again to induce anti-tumoral immunity. This immuno-gene therapy was started for Renal Cell Carcinoma in the common urology area, and in Japan, has been practiced as clinical study in gene therapy for Renal Cell Carcinoma by the Institute of medical Science Hospital, University of Tokyo, since 1998 (5). However, as for the autologous tumor cell-based vaccine for prostate cancer, the autologous tumor cells are difficult to collect and culture, so that a plurality of allogenic tumor cell line (LNCaP cell line and PC-3 cell line)-based vaccines have been proposed from the following year 1995 (4). The clinical trials in gene therapy using allogenic LNCaP cells with Interleukin-2 (IL-2) and Interferon-gamma genes (6), and GM-CFS gene (4) were approved in 1995 and 1997 respectively. The differences between immuno-gene

therapies using autologous vaccine and allogenic Vaccine are summarized in Table 1.

3.2. Gene transfer

Also, the clinical trials of *in vivo* gene therapy, in which a gene is transferred directly into a cancer cells in the body, unlike *ex vivo* gene therapy in which an *ex vivo* gene-transduced cell is incorporated into the body as immuno-gene therapy noted above, have been approved since 1995. In addition, although *ex vivo* gene therapy mainly adopted retrovirus vectors as introduced vector, *in vivo* gene therapy has mostly adopted adenovirus vectors in the light of the benefits like high transduction efficiency etc. The principal features of the vector system are summarized in Table 2.

3.3. Therapeutic gene

For the introduced therapeutic gene, various genes have been used, and the representative genes used for *in vivo* gene therapy include HSV-tk (Herpes Simplex Virus-thymidine kinase) gene (7), cancer suppressor gene p53, etc (8). In 1996 the clinical trial in patients with post-radiation locally recurrent prostate cancer using an adenovirus vector with HSV-tk gene by U.S. Baylor College of Medicine was approved (7). HSV-tk is an enzyme which converts a prodrug such as ganciclovir with no cytotoxicity into a metabolite toxic product. A cancer cell in which this gene is transferred and expressed is specifically killed by the administration of prodrug. The therapeutic gene which exert toxic effects by combination with a prodrug like HSV-tk gene are conventionally called Suicide Gene but sometimes called Metabolite Toxic Gene, and we personally consider the latter is more comprehensible name matching reality. Furthermore, an approximately same protocol as in the clinical trial in prostate cancer gene therapy by the Baylor College of Medicine was practiced as the first clinical trial in prostate cancer gene therapy in Japan by Okayama University Hospital, and its course was reported by Dr. Nasu *et al* (8). The clinical trial in prostate cancer gene therapy using an adenovirus vector with p53 gene was approved in 1997, which was based on the extended indications for precedent p53 gene therapy to lung cancer (9).

3.4. Tissue-specific promoter

The most significant consideration of gene therapy for cancer is how the target gene is specifically expressed in the cancer cell with high-efficiency. The clinical trial in prostate cancer gene therapy using a tissue-



Figure 1. Transverse CT scan depicts the L3 osseous metastatic lesion. A: Pre-treatment B: 30 days after treatment C: 180 days after treatment.

specific promoter, which is cancer-specifically activated, was approved in 1998. This clinical trial in gene therapy adopted an adenovirus vector Ad-OC-TK in which Osteocalcin (OC) promoter with specific promoter activity confirmed in primary tumor of prostate cancer and bone and lymphnode metastasis and HSV-tk gene were inserted (10). When OC promoter and HSV-tk gene are combined, HSV-tk is specifically expressed in prostate cancer cells but not in normal cells, and the normal cells are also not killed by administration of variciclovir, a prodrug. Thus improved safety of prostate cancer gene therapy by the tissue-specific OC promoter enabled direct injection of vectors into bone and lymph node metastasis as well as local prostate cancer. The same clinical study in gene therapy as in U.S. University of Virginia, Health Science Center have been practiced by the authors in Kobe University Hospital since 2003(11). Six patients were enrolled in this study, and all of them tolerated this therapy with no serious adverse events and remain alive at least 8 months. A PSA response was observed (the PSA level before treatment: 341.7 ng/ml → in 8 months: 4.9 ng/ml, and then the PSA level was increased again) with 12 months of time to PSA progression (TTP) in one patient. A prolonged TTP, 5 months was observed in another patient. Figure 1 shows a CT photograph of bone metastasis in a treatment site in a patient showing PSA response in this clinical study.

3.5. Replication-competent virus vector

In relation to the conventional vectors, replication-deficient virus vector which was genetically-modified so as not to replicate in human cells had been used from the safety aspect. The replication-deficient virus vectors are pointed to have the limitation in the transduction efficiency, and a clinical trial in prostate cancer gene therapy replication-selective virus vector which was replicated only in cancer cells (12) was therefore proposed in 1998. It was an adenovirus vector CN706, which became able to replicate only in prostate cancer cells by control of E1a gene required for adenovirus replication under the prostate cancer-specific PSA promoter (13). Also, a clinical trial in gene therapy using AD-5CD/Tkrep was approved in 1999 (14). The AD-5CD/Tkrep was prepared by simultaneously transferring two suicide genes HSV-tk and CD (Cytosine Deaminase) into a replication-competent adenovirus which became able to be replicated and to amplify only in cancer cells by partial deficiency of E1b gene required for adenovirus replication in normal cells.

4. FETAL CASE

Thirteen clinical trials in prostate cancer gene therapy were approved by the NIH and RAC in 1999, however only five clinical trials were approved in the following year 2000. The downward turn of the ever-increasing clinical trials resulted from an unfortunate accident in 1999. In September 1999, an 18 year-old boy Jesse Gelsinger died of the clinical trial in gene therapy in U.S. University of Pennsylvania (15). This affair dominated news headlines and developed into a major social problem. At the time, the congenital diseases due to single-gene

deficiency had been frequently studied as preferable indications for gene therapy. Ornithine transcarbamylase (OTC) deficiency was also supposed to be included, and *in vivo* gene transfer of the OTC gene into liver cells by an adenovirus vector was projected (16). Although some members initially pointed out risks of hepatic arterial infusion as a standard of the adenovirus vector administration, direct hepatic arterial infusion of adenovirus was eventually conducted at a concentration as high as 10^{13} p.f.u. (Plaque Forming Unit). As a result, Jesse Gelsinger died of multiple organ failure caused by systemic inflammatory response syndrome (SIRS) (17). In this case, it was criticized that the protocol in selecting patients was deviated and the patient was not previously appropriately informed of serious adverse effects observed in animal studies, which raised problems on the subsequent regulation and management in the clinical trials in gene therapy.

5. AFTER 2000

In and after 2000, immuno-gene therapies based on and cytokine genes such as GM-CSF, IL-2 and IL-12, and gene therapies using replication-competent adenovirus vectors have become the mainstream of the clinical trials in prostate cancer gene therapy. In 2004, a clinical trial in gene therapy using Allogenic Vaccine (GVAX) prepared by introducing the GM-CSF gene into allogenic tumor cell lines (LNCaP cell line and PC-3 cell line) proceeded to Phase III clinical trial for the first time in prostate cancer (18). This clinical trial is based on a method that Autologous Vaccine, which was prepared by introducing GM-CSF gene into the autologous tumor cell by retrovirus and approved as prostate cancer gene therapy for the first time in 1994, is developed and improved. For gene introduction of GM-CSF gene, Adeno-associated Virus (AAV) is used, and for the allogenic tumor cell line, two representative prostate cancer cell lines LNCaP and PC-3 are used. In the plan of this trial, a randomized open-label study with a group of chemotherapy drug docetaxel treatment will be conducted, and a total of 600 patients will be entered.

This paper simply outlined the 10 years of history of the clinical trials in prostate cancer gene therapy in U.S., allowing reconfirmation of a stream of technologic innovations in this field. The wave of practical applications of gene therapy for prostate cancer is closing in, and it is expected that this field will further make advance in the future.

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Abbreviations: ADA : adenosine deaminase; PSA: Prostate Specific Antigen; NIH: National Institute of Health; RAC: Recombinant DNA Advisory Committee; GM-CSF: granulocyte-macrophage colony stimulating factor; IL-2: Interleukin-2; HSV-tk: Herpes Simplex Virus-thymidine kinase ; OC: Osteocalcin; TTP: time to PSA progression; CD: Cytosine Deaminase; OTC: Ornithine transcarbamylase; p.f.u.: Plaque Forming Unit; SIRS: systemic inflammatory response syndrome

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