Award Number: W81XWH-04-1-0186

TITLE: A Novel Therapeutic System for the Treatment of Occult Prostate Cancer

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REPORT DATE: May 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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A Novel Therapeutic System for the Treatment of Occult Prostate Cancer

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The goal of this research was to investigate efficacy and mechanisms of H5BVIFN-β, a novel immunotherapeutic agent, against prostate cancer in animal models. The purpose of study in the first year was to determine efficacy of H5BVIFN-β therapy in suppressing progression of tumors of TRAMP-L5 murine prostate cancer cells in syngeneic immune competent mice. We found that intratumoral delivery of H5BVIFN-β significantly inhibited growth of both orthotopic and ectopic lesions of TRAMP-L5 cells. The H5BVIFN-β therapy moderately but significantly prolonged the survival of tumor-bearing mice. The HBVIFN-β therapy, however, could not eradicate TRAMP-L5 tumors in either the orthotopic or ectopic model.

Interferon-beta, lyophilized insect cells, immunotherapy, murine prostate cancer, orthotopic model, macrophages, interleukin-12

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A. INTRODUCTION

The specific aims of this research are unaltered from the original proposal. They remain to be investigating efficacy of our novel immunotherapy system in the treatment of occult prostate cancer and mechanisms by which this therapy suppresses tumor growth. The task for the first year is to determine the efficacy of the therapy.

I moved from University of Texas M. D. Anderson Cancer Center to University of Cincinnati College of Medicine during the last year (2004) and there are personal changes in my laboratory. We first established methods for preparing the therapeutic agent in my current institution. Our data show that H5BVIFN-β prepared in my current laboratory is similar or equivalent to those used in our preliminary studies. We then carried out therapy studies as proposed in the “Statement of Work” for year one. Progress is summarized below.

II. PROGRESS REPORT BODY

II.1. Preparation of therapeutic agent H5BVIFN

H5 insect cells in serum-free insect cell growth medium (2 x 10⁵/ml) were infected with different doses (0-100 multiplicity of infection, MOI) of a baculovirus vector encoding mouse interferon-β (BV-IFN-β). Culture supernatant was removed and the infected cells were harvested at 24-96 hr after the infection, counted, and lyophilized. Mouse IFN-β in the cell lysates was measured by enzyme linked immunosorbent assay (ELISA). In four independent preparations, H5 cells infected with 10 MOI of BVIFN-β for 72 hr produced 670 +/- 15 ng/10⁶ cells of intracellular IFN-β based on ELISA. The presence of IFN-β in the lysates was confirmed by western blot analysis and IFN-β activity in the lysates was determined by using a macrophage-activation based bioassay established in our previous studies [1]. Recombinant mouse IFN-β was used as positive control in all assays. Based on these analyses, we defined 1 x 10⁶ lyophilized H5BVIFN-β as one therapeutic unit that contains approximately 2 x 10⁴ units of biologically active mouse IFN-β.
II.2. To investigate efficacy of intratumoral injection of H5BVIFN-β in suppressing growth and metastasis of TRAMP-L5 tumors in immune-competent syngeneic mice
a. To investigate therapeutic efficacy of intratumoral injection of H5BVIFN-β against TRAMP-L5 tumors
a.1. Doses of H5BVIFN-β needed to eradicate orthotopic TRAMP-L5 tumors.

TRAMP-L5 cells (10^5/mouse) were injected into the prostate of male C57BL/6 mice. Seven to 10 days after the orthotopic implantation, when the tumors reach approximately 50 mg, mice were intralesionally injected with 0.5, 1, or 2 units of H5BVIFN-β. Tumors injected with PBS, 4 x 10^4 units of IFN-β or 2 x 10^6 lyophilized H5 cells served as controls. The experiments were terminated 2-3 weeks after the therapies when mice in control group became moribund. The prostate tumors were removed and weighed. As shown in Fig. 1, a single intratumoral injection of H5BVIFN-β inhibited tumor growth in a dose-dependent manner. Injection of H5 or IFN-β did not significantly alter growth of TRAMP-L5 tumors. Treatment with 2 units of H5BVIFN-β suppressed tumor growth by approximately 50%.

However, this therapy was not able to eradicate tumors in any treated mice.

To determine whether multiple injections of H5BVIFN-β could eradicate TRAMP-L5 tumors, we carried out the therapy studies in the subcutaneous tumor model of TRAMP-L5 cells as proposed in “Anticipated results and alternative approaches”. TRAMP-L5 cells (10^5/mouse) were inoculated into the subcutis of male C57BL/6 mice.
When tumors reached 4-5 mm in diameter, they were injected with 2 units of lyophilized H5BVIFN-β once, once a week, or two times a week until experiments were terminated. Tumors injected with PBS or H5 cells (two times a week) served as controls. The subcutaneous tumors were measured every other day with calipers and mice were sacrificed when the tumors reach 20 mm in diameter. Data in Fig. 2 show that a single injection of H5BVIFN-β had no significant effect on the growth of TRAMP-L5 tumors. Growth of the subcutaneous tumors was significantly retarded when they were injected once a week and more significantly two times a week with lyophilized H5BVIFN-β, but not PBS or lyophilized H5. However, the therapies did not completely destroy the tumor in any mouse.

![Graph showing tumor growth](image)

**Fig. 2. Effects of H5BVIFN-β therapy on growth of TRAMP-L5 tumors in the subcutis of C57BL/7 mice.*** and ***, p<0.05 and p<0.01, respectively, in comparison with tumors treated injected with lyophilized H5 cells or IFN-β.

a.2. Effects of H5BVIFN-β therapy on existing lymph node metastases.

We proposed to investigate efficacy of H5BVIFN-β therapy against existing lymph node metastases if we find a dosing of H5BVIFN-β that can eradicate primary tumors. Since we failed to reach that goal in both orthotopic and ecotopic models, we did not carry out this experiment.
a.3. Effects of H5BVIFN-β therapy on the survival of tumor-bearing mice.

TRAMP-L5 tumor cells were inoculated into the prostates of C57BL/6 mice. Seven days later, tumor-bearing mice were divided into 4 groups and treated by intratumoral injection of PBS, $2 \times 10^6$ lyophilized H5 cells, $4 \times 10^4$ units of IFN-β, or 2 units of H5BVIFN-β. Mice were monitored daily and sacrificed only when they became moribund. Effects of the treatments on the survival of tumor-bearing mice were analyzed by Kaplan-Meier method shown in Fig. 3. Median survival times for mice injected with PBS, H5 cells, IFN-β, and H5BVIFN-β were 37, 37, 38, and 44 days, respectively. Only the injection of H5BVIFN-β significantly prolonged survival of tumor-bearing mice ($p<0.01$ in comparison with those injected with PBS). We are currently performing another experiment to repeat the survival study.

![Fig. 3. Effects of H5BVIFN-β on survival of mice-bearing orthotopic TRAMP-L5 tumors.](image)

b. To determine whether mice cured of primary tumors are resistant to a second challenge of TRAMP-L5 cells.

Since we failed to eradicate primary tumors, we could not carry out the experiments proposed in this section.

c. Recommended changes in proposed research
In previous studies, we showed that the intratumoral injection of H5BVIFN-β into established subcutaneous lesions of UV-2237m mouse fibrosarcoma and K-1735M2 melanoma eradicated both primary tumors and preexisting lung or brain metastases. This therapy also conferred long-term tumor-specific protection in mice cured of primary tumors. The therapeutic effects of the lyophilized H5BVIFN-β relied on both CD4+ and CD8+ T cells and were diminished in nude mice and mice depleted of CD4+ and CD8+ T-cells [2,3]. In this TRAMP-L5 tumor model, we found, however, that H5BVIFN-β only partially suppressed tumor growth. Although the results are publishable, they are less than desired. TRAMP-L5 cells were derived from TRAMP-C2 cells through 8 cycles of implantation into the prostate (3 times re-cultured from prostatic tumors followed by 5 times re-cultured from lymph node metastases). TRAMP-L5 cells grow much faster than TRAMP-C2 cells both in culture (the doubling times of TRAMP-L5 and TRAMP-C2 are 19 hr and 48 hr, respectively) and in mice. One of possible reasons for the failure of H5BVIFN-β therapy in eradicating TRAMP-L5 tumors is that the primary tumors grow too fast. We, therefore, propose to evaluate efficacy of H5BVIFN-β therapy against TRAMP-C2 tumors that can be controlled by immunotherapies as reported by others [4,5]. In this experiment, TRAMP-C2 cells (10^6/mouse) will be injected into the prostate or subcutis of C57BL/6 mice. When tumors reach 50 mg (prostatic tumors) or 4-5 mm in diameter, they will be injected with PBS, H5, or H5BVIFN-β. Experiments will be terminated when mice in any treatment group become moribund (prostatic tumors) or when tumors reach 20 mm in diameters (subcutaneous tumors). If we found that H5BVIFN-β therapy can eradicate or produce much greater growth inhibitory effects on TRAMP-C2 tumors, we shall perform the mechanistic studies proposed in the third task (the third year) of “Statement of Work” in TRAMP-C2 model.

III. KEY ACCOMPLISHMENT

My laboratory was relocated to a new institution in the last year. I have trained a new postdoctoral fellow and a new research assistant to carry out the research proposed. We have re-established the therapeutic system in my current institution and evaluated the efficacy of H5BVIFN-β therapy against occult TRAMP-L5 tumors in C57BL/6 mice. We found that a single intratumoral injection of H5BVIFN-β could suppress the growth of
orthotopic TRAMP-L5 tumors by approximately 50% and moderately prolonged the survival of tumor-bearing mice (by 7 days or 19%). A therapy with multiple injections of H5BVIFN-β significantly retarded growth of TRAMP-L5 tumor in the subcutis of C57BL/6 mice.

IV. REPORTABLE OUTCOMES
No reportable outcomes have yet to arise from this project.

V. CONCLUSIONS
1. The intratumoral injection of H5BVIFN-β significantly inhibited growth of both orthotopic and ecotopic lesions of TRAMP-L5 cells.
2. The H5BVIFN-13 therapy moderately but significantly prolonged the survival of tumor-bearing mice.
3. The HBVIFN-β therapy could not eradicate TRAMP-L5 tumors in either the orthotopic or ecotopic model.
4. Our data suggest that intratumoral injection of H5BVIFN-β could be a potential novel therapy for human prostate cancer.
5. In the next year, I propose to also study efficacy of H5BVIFN-β therapy against TRAMP-C2 tumors. If we found that H5BVIFN-β therapy can eradicate TRAMP-C2 tumors or produce greater growth inhibitory effects, we shall perform the mechanistic studies proposed in the third task (the third year) of “Statement of Work” in TRAMP-C2 model.

VI. REFERENCES