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The long-term goal of the proposed study is to develop a genetic immunotherapy strategy that can compliment and enhance current prostate cancer radiotherapy. The specific strategy that will be adopted in this proposal is to test the capacity of an adenovirus encoding immunostimulatory genes IL-12 and B7 to enhance the therapeutic effects of ionizing radiation in an experimental murine prostate tumor model TrampC. A substantial progress has been made towards this goal. We have now conducted experiments where we combined radiotherapy and genetic immunotherapy in the TrampC1 model. The results indicate synergistic effects in controlling subcutaneous tumor growth. We have also developed a murine prostate cancer model where tumor growth can be imaged non-invasively through a innovative bioluminescence imaging system. We anticipate this model will greatly facilitat our future studies on prostate cancer metastases.

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Year 1 progress report

The long-term goal of the proposed study is to develop a genetic immunotherapy strategy that can compliment and enhance current prostate cancer radiotherapy. The specific strategy that will be adopted in this proposal is to test the capacity of an adenovirus encoding immunostimulatory genes IL-12 and B7 to enhance the therapeutic effects of ionizing radiation in an experimental murine prostate tumor model TrampC. To achieve the long-term goal, the following specific tasks were planned:

Task 1. To test the efficacy of an adenovirus encoding immunostimulatory IL12 and B7 genes (AdIL12.B7) to enhance the radiation treatment of primary tumors in a mouse prostate cancer model. (Months 1-18).

Task 2. To examine the potential capacity of combined radiotherapy and AdIL12.B7 mediated genetic immuotherapy to control distant metastases (Months 12-24).

Task 3. To elucidate the mechanisms of potential anti-tumor effects of the AdIL12.B7 (Months 18-36).

We have made steady progress towards our stated goals:

For task 1, we have finished the first experiments to test the efficacy of the combined genetic immunotherapy with radiation therapy. Our results indicated the following:

1) There is synergistic efficacy when AdIl12.B7 is combined with radiation therapy. The growth delay achieved when the two modalities were combined were greater than the sum of growth delay achieved when each modality was applied individually;
2) The synergistic effects were observed when AdIL12.B7 virus was injected into tumor after radiation therapy. Best results were obtained when the gene therapy vectors were injected 1-3 days after the completion of the radiation therapy. When the virus vectors were injected before radiation therapy, no increased effects were seen at all. These results may be explained by the sensitivity of the immunoeffector cells to radiation.
3) The above results suggest an approach for future combination of genetic immunotherapy with radiation therapy.

For task 2, in order to monitor tumor metastasis non-invasively, we are developing a novel firefly luciferase-based model for observing metastatic tumor growth. We have established a stable prostate cancer cell line that expresses the luciferase gene. This cell line has been tested in syngeneic C57BL/6 mice Figure 1 shows tumor growth in vivo. We are very pleased with the results because it gives us the ability to monitor metastatic tumor growth non-invasively. Previously, in order to monitor metastatic tumor growth, we would have to inject the tumor cells and wait long periods of time until mice appear moribund. We would then dissect the animals and look for signs of tumor growth in major organs such as lung and liver. With this new model, we just need to anesthetize the animals, inject the luciferin substrate, and image tumor growth by use of the Xenogen Imaging system(1-3).

Work is underway to evaluate the potential to use this new model in monitoring tumor growth after radiation and gene therapy.

Figure 1. TrampC1 tumor growth as observed through luciferase imaging. Tramp-C1 cells stably transduced with the firefly lucifrease gene was injected subcutaneously ($1x10^6$ cells per animal). They were then imaged daily after injection of luicferin. Imaging was carried out by use of the Xenogen light imaging system. Shown here is the image taken at day 6 after injection.

For task 3, work has not begun. We anticipate our work will begin on task 3 in the next 6 months.

Reportable Outcome:

- We have conducted experiments that indicated a synergistic interaction between radiation therapy and genetic immunotherapy. Administration of immmunostimulatory IL12 and B7-encoding adenovirus after radiation therapy can significantly boost the efficacy of radiation therapy in a murine prostate cancer model.

- We have established a mouse prostate cancer model, which allows for tumor growth to be imaged non-invasively. This model can greatly facilitate the study of prostate cancer metastasis, because prostate cancer growth in this model can now be monitored non-invasively and sensitively (as few as 1000 cells can be imaged).

Conclusions:

We have made steady progress in the proposed project. In addition, we have added some novel elements (e.g. luciferase-based imaging) so that planned experiments can be conducted with better monitoring approach. We expect to accomplish the goals stated in the proposal on time.

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