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## INTRODUCTION

As a DOD-PCRFP postdoctoral fellow training award recipient I have gained a wealth of knowledge and research experience in gene therapy and prostate cancer research during the period of December 2001 to December 2002. I routinely join the journal club and participate in the weekly laboratory and summary meetings during which the entire lab discusses new developments and experimental results. I have also been encouraged by mentor Dr. O'Malley to attend relevant scientific conferences to learn new information related to my projects.

In research project, I have carried out two tasks as proposed in the Statement of Work to establish a regulable system for applying on the prostate cancer gene therapy. One is to generate regulatory components suitable for control of transgene expression in prostate cancer cells (Task 1), and the other one is to test the target gene (therapeutic gene) induction and anti-cancer functions in cells and animals (Task 2).

## BODY

### *Task 1. To construct and test a prostate-specific, inducible transgene expression system.*

The effort has been put to modify the regulatory component GLp65 for optimal control and expression of transgene in target cells. The construction and modification were involved in replacement of a hinge region of PR-LBD in GLp65 with a recombinant GR-LBD sequence. The resulted new regulator G2Lp65 was demonstrated by using a green fluorescence protein (GFP)-tag for subcellular tracking that the distribution of the fusion molecules was changed as designed. G2Lp65 has lower basal activity in absence of the exogenous inducer Mifepristone and has higher induction fold in presence of inducer (Fig. 1). In additions, G2Lp65 and its derivative G3Lp65 have relatively higher dose-response in certain range ( $10^{-11}$ ~ $10^{-9}$  M) of inducer (Fig. 2). These improved features make the regulator more versatile for various gene therapy applications. It also allow us in future to make fine adjustment of transgene expression level to fit into the therapeutic window for cytokines and other systemic toxicity-sensitive gene products in prostate cancer therapy.

The effort has also been on generation of a tissue-specific regulator. This new regulator was derived from a recombinant androgen receptor (AR) by replacement of DNA-binding domain and point mutagenesis. The resulted regulator ARG65 can not bind on the AR responsive elements (ARE) to activate AR understream genes, but instead it may have a dominant negative effect on the endogenous AR by forming heterodimers. Although this dominant negative effect appears rather weak in LNCaP cells, it still could be inversely benefit for prostate cancer patient due to partially inhibition

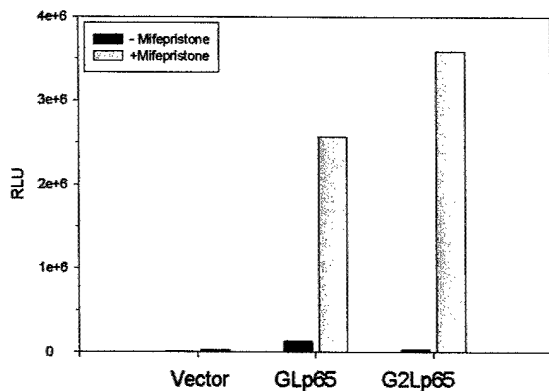


Fig. 1. Mifepristone-dependent transactivities of regulators GLp65 and G2Lp65.

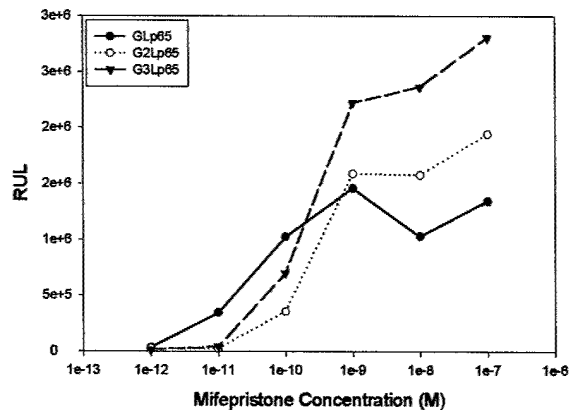


Fig. 2. Mifepristone dose-dependent transactivities of regulators GLp65, G2Lp65 and G3Lp65.

of androgen-dependent tumor growth. More importantly, ARG65 positively responds to a low level of androgen and has a significant transactivity on an exogenous promoter-mediated transgene expression in prostate cancer cells such as LNCaP cells (Fig. 3). Because this regulator uses native coactivators and ligand existed in prostate, its transactivity can reach as high as three to four magnitudes as shown in the assays (Fig. 3). Also, because the activity can sustain in prostate without additional inducer, providing a convenience for chronic disease treatment. However, if necessary, the induction level can be fine-tuned by administration of non-steroid anti-androgen drug (Fig. 3), therefore possibly to achieve optimal therapeutic window for prostate cancer therapy.

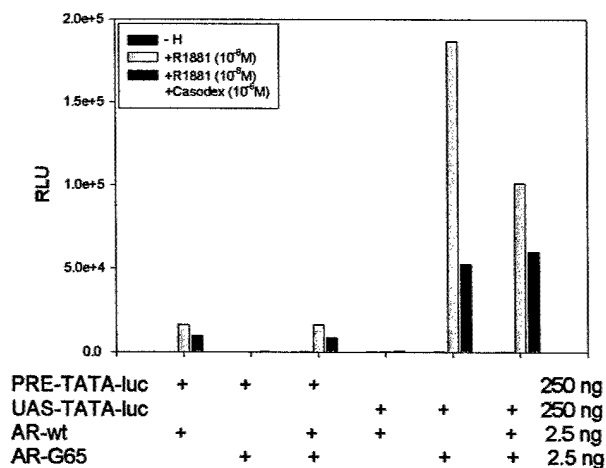


Fig. 3. Androgen-dependent transactivities of regulator ARG65 in LNCaP cells.

**Task 2. To analyze functional properties of inducible gene products *in vitro* and *in vivo*.**

Currently, my effort is to test effectiveness of target genes in conjunction with regulatory system for inhibition of cancer growth. Among the selected anti-angiogenic genes as proposed in project, interferon gene construction and testing are still in process, whereas the endostatin gene construct has been completed and examined carefully in our system. To produce a secretory form of endostatin, a secretion signal-containing endostatin gene was subcloned into a GLp65-derived regulable expression module in adenoviral vector and subsequently packaged into helper-dependent adenovirus for gene delivery. The expression assay showed that endostatin can be significantly induced in transfected cells by addition of Mifepristone, and its protein concentration can reach over 1000 ng/ml (Fig. 4). Use of this endostatin protein preparation for the matrigel implantation assay in mice, the results showed that new blood vessels in matrigels were apparently reduced in comparison with controls, suggesting that the endostatin produced from our inducible expression system has the anti-angiogenic bioactivity. To achieve inducible production of endostatin in mice, the recombinant adenovirus was delivered via mouse tail vein, and then 7 to 10 days later the inducer Mifepristone was administrated. Endostatin quantitative assay showed the serum levels of endostatin increased sharply after administration of inducer, the peak appeared on the first week period and then the levels decreased over the time (Fig. 5).

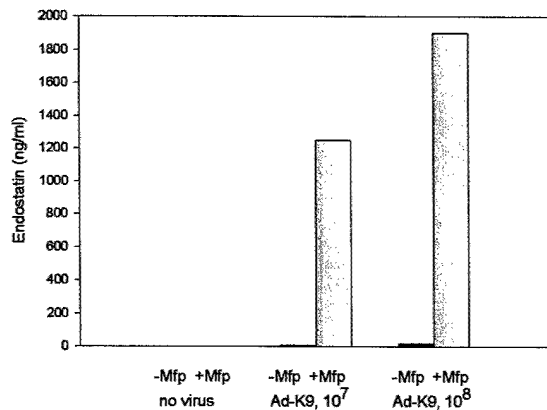


Fig. 4. Induction of endostatin in cells transfected by recombinant adenovirus Ad3.51ES.

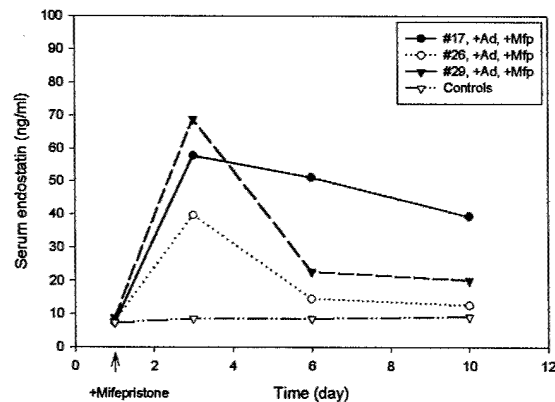


Fig. 5. Induction of endostatin in mice transfected by recombinant adenovirus Ad3.51ES.

Using the established endostatin expression system, two different mouse tumor models were examined for endostatin anti-tumor functions. One was transgenic hepatocellular carcinoma model from J. Butel's laboratory and the other one was the transplanted prostate reconstitution tumor model from T. Thompson's laboratory. The liver tumor model was not proposed in original Statement of Work.

Use of this model was for the proof of principle purpose for endostatin, because adenovirus can concentrated in liver and therefore achieve higher local concentration of endostatin. The results showed that endostatin was able to partially inhibit hepatic tumor growth in the liver tumor model (Table 1). However, the endostatin produced by the same inducible system did not showed a significant inhibitory effect on the prostate tumor growth based on tumor measurement (Fig. 6). Further test and histological analysis are still in process.

Table 1. Effect of endostatin on mouse liver tumor growth

Group	total	Genotype	Ad-ES	Mfp	Body (B)	Liver (L)	L/B-Ratio
I	9	wt	-	-	30.81 ±3.53	1.76 ±0.25	0.06 ±0.005
II	9	Tg	-	-	33.96 ±4.67	5.17 ±1.36	0.15 ±0.028
III	10	Tg	+	-	34.80 ±5.52	6.51 ±2.99	0.18 ±0.055
IV	9	Tg	+	+	32.71 ±4.55	4.34 ±1.43*	0.14 ±0.018*

\*P<0.05 between groups III and IV.

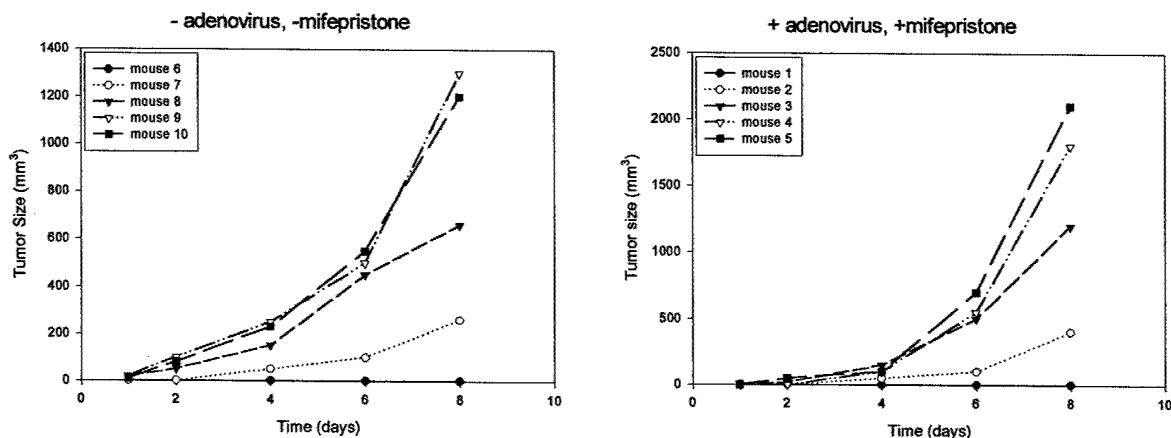


Fig. 6. Effect of endostatin on the transplanted mouse prostate tumor growth.

## KEY RESEARCH ACCOMPLISHMENTS

1. Two new regulators for inducible therapeutic gene expression have been generated. These regulators further improved the inducibility and sensitivity of our system and made them more suitable for the prostate cancer gene therapy.

2. An adenoviral construct of endostatin and its help-dependent packaged adenovirus have been generated and tested in cell cultures, in matrigel assays and in two mouse tumor models. Although endostatin in our current system appeared rather weak to block tumor growth, the regulatory system for endostatin expression was proved to be very effective.
3. The vectors and protocols for target gene functional analysis in conjunction with the adenovirus-mediated gene delivery and the transgene induction has been developed. This will facilitate the next period of studies on the other candidate therapeutic genes.

### **REPORTABLE OUTCOMES**

No reportable outcome in this period.

### **CONCLUSIONS**

Regulable gene therapy represents a new strategy to control and amplify efficacy of therapeutic gene. By the research efforts during this first period, the modified Mifepristone-inducible transgene expression system has been successfully integrated into helper-dependent adenoviral vector for the pre-clinical study of the prostate cancer gene therapy. More studies as proposed will be put into functional test of anti-tumor gene suitable for prostate cancer therapy in next research period.

### **REFERENCES**

None.

### **APPENDICES**

None.