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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

This report summarizes the first year of activity on this study. The **long-term goal** of this project is to better understand why some prostate tumors grow aggressively while others are extremely slow growing lesions. The **objective** of the proposed research is to establish immortalized stromal cell lines derived from normal human prostate and from human prostate cancer and to use these cells to investigate the role of IGFs in prostate cancer growth. In this year we have focused on generating the molecular tools which are needed to progress with the proposed work. Retroviral vectors for the introduction and selection of all of our genes of interest (telomerase, Insulin-like growth factors and IGF binding protein 3) have been made and validated. Generation of immortalized stromal cell lines is underway and these cells are in the process of being characterized as they are generated. No major technical obstacles have cropped up. The project is close to its timeline predicted in the accepted statement of work.

14. SUBJECT TERMS:
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Introduction

The **long term goal** of this project is to better understand why some prostate tumors grow aggressively while others are extremely slow growing lesions. The **objective** of the proposed research is to establish immortalized stromal cell lines derived from normal human prostate and from human prostate cancer and to use these cells to investigate the role of IGFs in prostate cancer growth. The **central hypothesis** on which this proposal is based is that prostate cancer progression is regulated, at least in part, by paracrine interactions between the prostatic stroma and the tumor. The first specific aim will generate immortalized cell lines with which to pursue mechanistic studies. The **hypothesis** is that fibroblastic cells immortalized by the insertion of a telomerase (hTERT) construct will behave in the same way in bioassays of their tumor-promoting activity as do the primary cell cultures from which they are derived. The **rationale** for these experiments is based upon observations by the PI and others on the role of stromal cells as promoters of carcinogenesis. The **hypothesis** of the second specific aim is that IGF family ligands act in a paracrine manner to elicit proliferation and/or tumorigenesis in human prostate cancer. The **rationale** for this specific aim is based on a variety of published observations connecting local and systemic levels of IGFs with prostatic growth and malignancy. The third specific aim will examine gene regulation in epithelial cells caused by changes in IGFs in the local microenvironment. The **hypothesis** is that changes in epithelial behavior are reflected in gene expression, the **rationale** is to identify gene products which might be targets for theraputic intervention.

Statement of Work
Paracrine Regulation of Prostatic Carcinogenesis

Task 1
Establish and characterize immortalized normal and carcinoma associated human prostatic fibroblast lines.

a. Establish retroviral expression of hTERT in LZRS/Phoenix A cells (month 1)

Transfection of LZRS construct into Phoenix A packaging cells. Selection of stable transfectants.

b. Infect fibroblasts and select based upon reporter gene expression (months 2-4)

Infection of fibroblasts, FACS sorting for expression of GFP reporter

c. Screen hTERT expressing cells for malignant transformation (months 3-9)

Graft to athymic mouse hosts for 3 months, histopathological examination of recovered grafts (total 36 mice).

d. Establish cell activity in tissue recombination bioassays (months 3-9)

Recombine fibroblast cell lines with BPH-1 reporter cells. Graft to athymic mouse hosts, examine recovered grafts to determine biological effects (total 36 mice).

This task will produce immortal fibroblastic cells representative of both normal and malignant human prostate.

Task 2
Investigate the role of insulin-like growth factors in prostate tumor progression and proliferation.

a. Generate LZRS constructs containing IGF-1, IGF-2 and IGFBP-3 and EYFP reporter (months 6-12)

The constructs will be made from already existing pieces

b. Establish retroviral expression of IGF family members in LZRS/Phoenix A cells (months 9-15)

Transfect LZRS constructs into Phoenix A packaging cells. Select stable transfectants

c. Infect immortalized stromal cells with the IGF family-expressing retroviruses (months 10-18)

d. Select fibroblasts expressing EYFP reporter (months 11-19)

FACS sorting for the EYFP reporter

e. Screen infected cells for malignant transformation (months 12-22)

Graft to athymic mouse hosts for 3 months, histopathological examination of recovered grafts (36 mice).

f. Assess biological activity of IGF family-expressing cells in vitro (months 16-26)

In vitro conditioned medium experiments

g. Assess biological activity of IGF family-expressing cells in vivo (months 16-30)

Recombine with BPH-1 cells, graft to nude mice, after three months recover grafts and undertake histopathological analysis (138 mice).

This task will provide a series of stromal cell lines expressing IGF-1, IGF-2 or IGFBP-3. These will be matched with cells which do not express these proteins. It will provide information on the role of IGF family members as mediators of prostatic carcinogenesis in vivo.

Task 3
Investigate changes in epithelial gene expression elicited by IGF family members in the stroma.

a. Make and graft tissue recombinants (months 24-32)

Recombine representative cell lines from specific aim 2 with BPH-1 cells. Graft and harvest grafts after three months.

b. Prepare RNA, make cDNA, hybridize to arrays (months 27-35)

Dissociate harvested grafts, sort cells. Prepare RNA from the epithelial cell population.

c. Analyze array data (months 28-36)

This task will provide data on the changes of gene expression induced in human prostatic epithelial cells growing in vivo by local changes in IGF ligand availability.

Work Ongoing and Completed

Task 1a. hTERT constructs have been made and inserted into LZRS retrovirus (see construct map in figure 1). The sequence of the construct has been checked. The ability of the retrovirus to infect human primary prostatic cell cultures has been confirmed. A variation of the proposed methodology was used in that a construct containing puromycin resistance was used in place of the selectible EGFP marker. (see fig 1) This modification makes cell selection more rapid and cost effective.

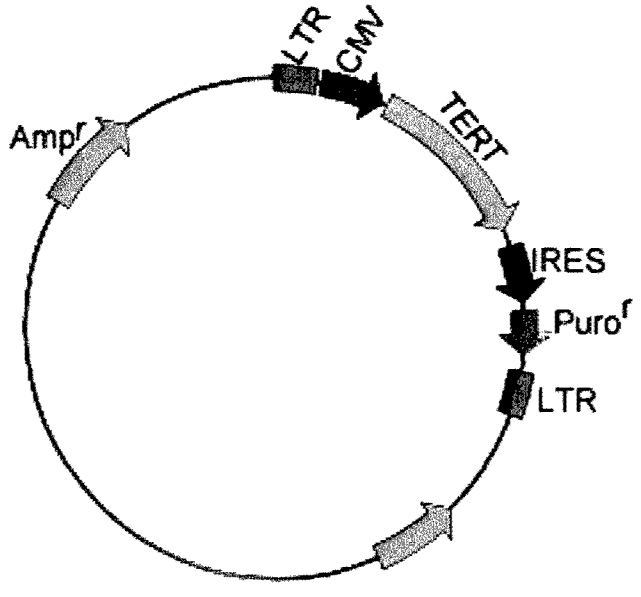


Figure 1. LZRS retroviral vector generated to allow expression of telomerase in human cells. The hTERT fragment is expressed under the control of the constituitively active CMV promoter. A puromycin resistance sequence is coexpressed with hTERT using an internal ribosomal entry sequence (IRES) to allow biochemical selection of cells expressing hTERT.

Task 1b. A series of normal and cancer associated prostatic stromal cell lines have been infected with the hTERT construct and puromycine selected. A real-time RT-PCR assay has been established to monitor and quantitate hTERT expression in the infected cell lines. As a check on protein expression a Western blot assay for hTERT expression is also being optimized.

Task 1c. Screening for malignant transformation caused by the specific retroviral insertion point is ongoing. As noted in the original application this is more of a theoretical than a practical concern, however this is an aspect of retroviral immortalization which must be formally tested before further experiments can be performed. To this point no malignant transformation has been observed.

Task 1d. Testing of the CAF/normal fibroblastic activity of the hTERT immortalized cells in a BPH-1 tissue recombination bioassay is also ongoing. This process is somewhat delayed as tissue recombinations cannot be performed with specific stromal cell strains until malignant transformation testing proves negative (task 1c). As more cells strains pass through this barrier the backlog is expected to ease.

Task 2a. LZRS constructs for expression of IGF-1, IGF-2 and IGF-BP3 have been generated. Construct maps shown in figure 2. The constructs are fully sequenced and validated.

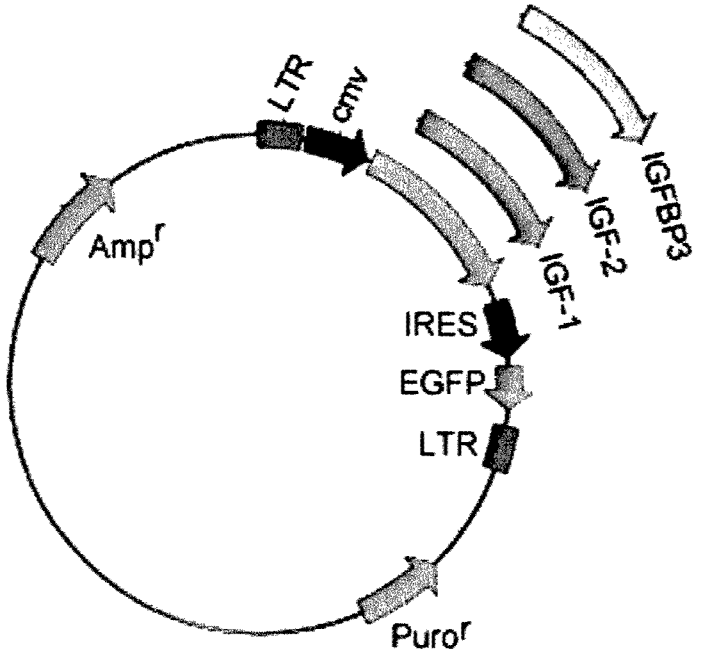


Figure 2. LZRS retroviral vector generated to allow expression of IGF ligands and IGFBP3 in human cells. The genes of interest are expressed under the control of the constituitively active CMV promoter. An enhanced green fluorescent protein sequence is coexpressed with the gene of interest using an internal ribosomal entry sequence (IRES) to allow FACS selection of cells expressing IGF family members.

Task 2b. Retroviral expression of the IGF-family LZRS retroviruses in PHNX cells has been successfully achieved. Transfected cells show expression of EGFP as expected.

Task 2c. Infection of human prostatic stromal cells with all three IGF family members has been achieved in limited numbers of cells strains at this point. Cell selection using FACS sorting is proceeding. Expression of GFP is confirmed in many cells. Cell sorting (see example on following page) allows successful separation of expressing and non-expressing cells. Post selection culture demonstrates expression of GFP in all cells (figure 3)

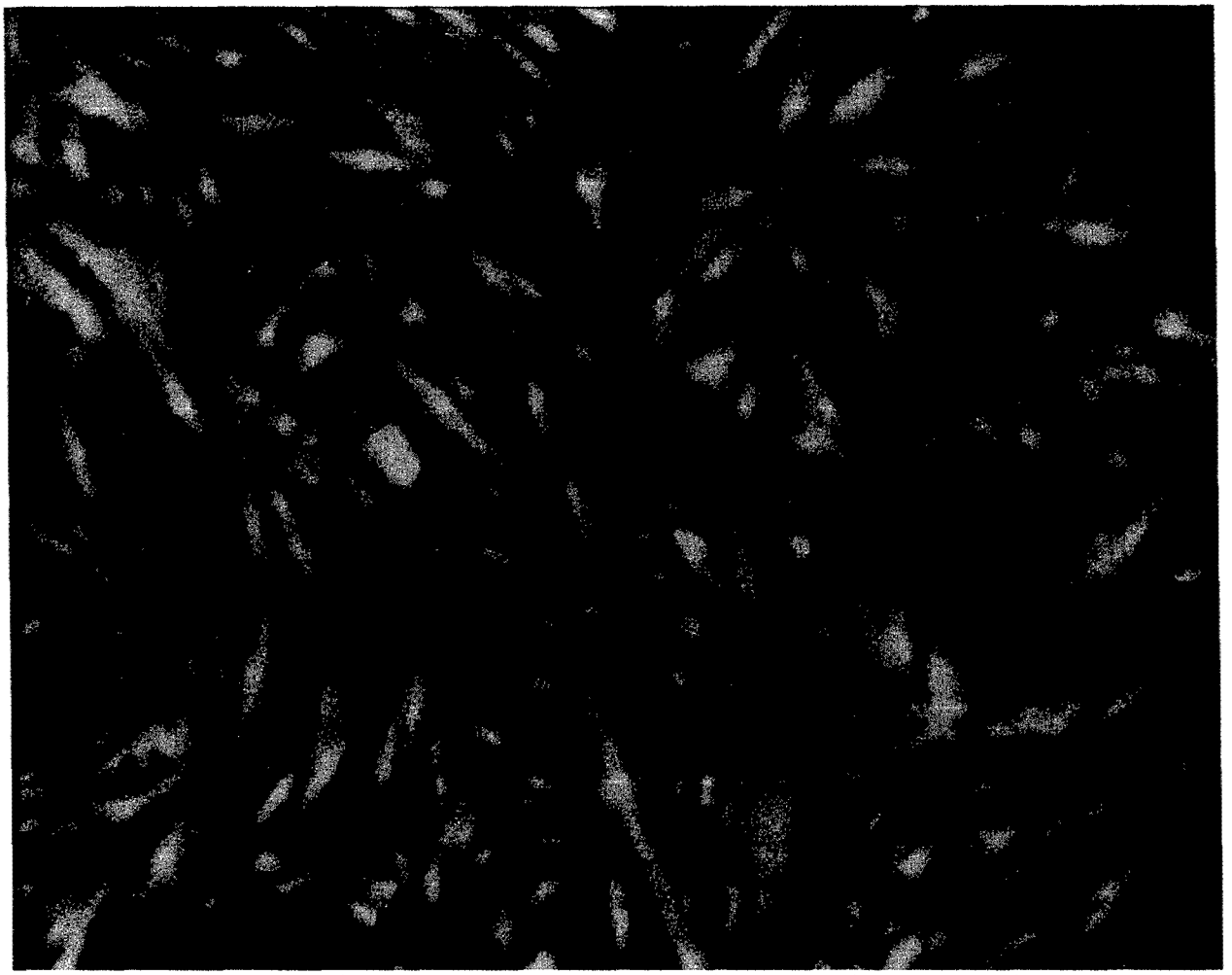
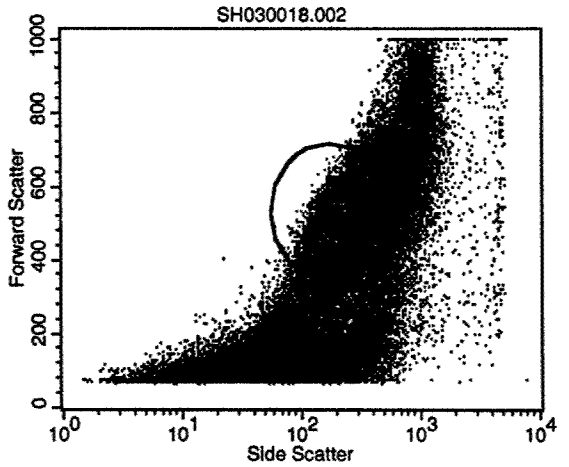
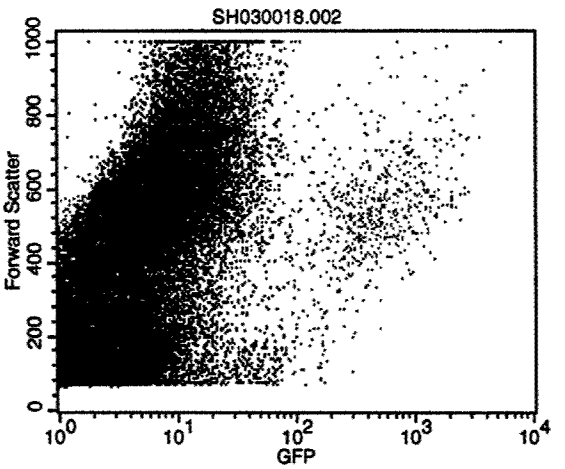


Figure 3. EGFP expression in IGF-1 retrovirally-infected FACS selected human prostatic fibroblasts. Following FACS selection essentially all cells express the reporter genes.



File: SH030018.002 Sample ID: NP5.3
Patient ID: IGF1 GFP Acquisition Date: 03-Jan-03
Gate: No Gate

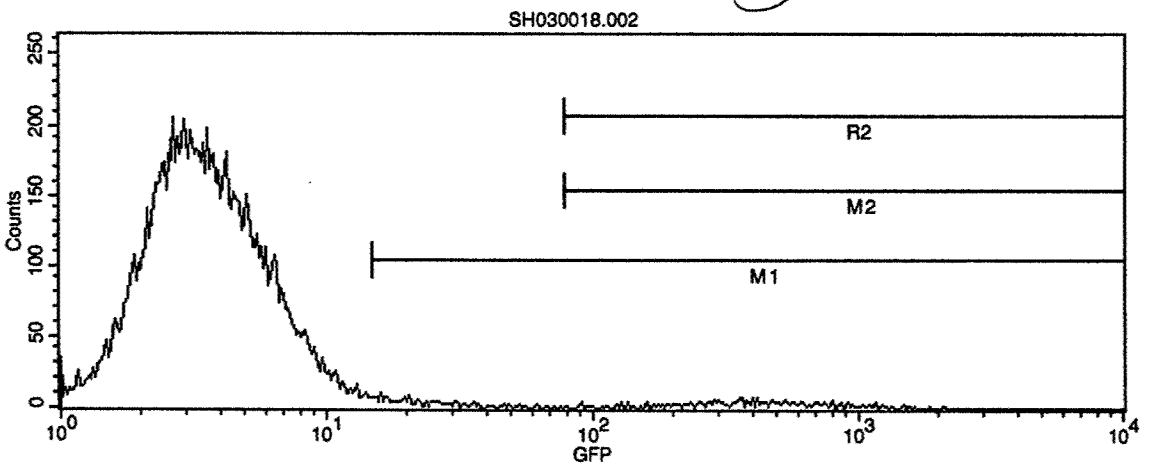
Region	Events	% Total	Px,Py
R1	25000	36.17	2, 1
R2	728	1.05	3, 0



File: SH030018.002 Sample ID: NP5.3
Patient ID: IGF1 GFP Acquisition Date: 03-Jan-03
Gate: No Gate

Gate	Events	% Gated	% Total
Sort	402	0.58	0.58

Sort=R1&R2 : 5 x 10^3 cells recovered



File: SH030018.002 Sample ID: NP5.3
Patient ID: IGF1 GFP Acquisition Date: 03-Jan-03
Gate: G1

Marker	Left, Right	Events	% Gated	% Total	Mean	CV	Peak Ch
All	1, 9910	25000	100.00	36.17	11.82	604.63	2
M1	15, 9910	681	2.72	0.99	292.25	111.76	15
M2	78, 9910	402	1.61	0.58	477.82	65.03	349

Page 1

Task 3. Not yet started

Key Research Accomplishments

- Establishing and validating of biochemically selectable retroviral vectors for the introduction of hTERT into primary cell cultures of human prostatic stromal cells.
- Establishing and validating of optically selectable retroviral vectors for the introduction of IGF-1, IGF-2 and IGFBP-3 into primary cell cultures of human prostatic stromal and epithelial cells.
- Confirmation of expression of introduced genes of interest in infected cell cultures.

Reportble Outcomes.

Book Chapter

Ishii, K. and Hayward, S.W. The History of Tissue Recombination Technology: Current and Future Research. In: Challenges in Prostate Cancer II. Bowsher, W. (Ed.) Blackwell, London (in press)

Conclusions.

This work is proceeding on the predicted timeline. No major hurdles have been encountered. The first year of this project, as described in the approved Statement of Work, was aimed at generating the molecular tools needed to proceed with the project. These tools are now in hand and the major experimental section of the project is now starting.