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14. ABSTRACT

In our previous in situ hybridization studies of prostate tissue arrays, RegIV was shown to be strongly expressed by a majority of metastatic hormone refractory tumors (HRPC) and weaker RegIV expression was found in a subset of primary tumors, but not expressed by normal tissue. Our goal is to better understand the role of RegIV in prostate cancer progression and to determine its possible use as a diagnostic marker for early and metastatic prostate cancers. We have developed and affinity purified a polyclonal anti-RegIV that has successfully been tested by immunohistochemistry on positive and negative controls. *In vitro* studies show cells overexpressing RegIV have a growth advantage and we have successfully made two si-RegIV constructs to knock down RegIV RNA and protein expression and *in vivo* studies are currently in progress.

15. SUBJECT TERMS
metastatic prostate cancer, polyclonal antibody, siRNA, immunohistochemistry

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INTRODUCTION

New markers for aggressive and potentially metastatic prostate cancer are needed. To test the role of Reg IV, a secreted protein expressed in most metastatic prostate cancers, as a tissue and serum marker, we will determine whether the expression of Reg IV in primary tumors correlates with the risk of recurring and developing progressive disease after surgery. Second, we will determine the role of Reg IV in progression to androgen independence and/or metastasis. Finally, if we find a role for Reg IV in either of these processes, we will ask if neutralizing antibodies against Reg IV can be used therapeutically in animal models.

PROGRESS REPORT

Aim 1: *To determine if Reg IV expression in primary tumors correlates with risk of recurrence or PSA-doubling time after recurrence*

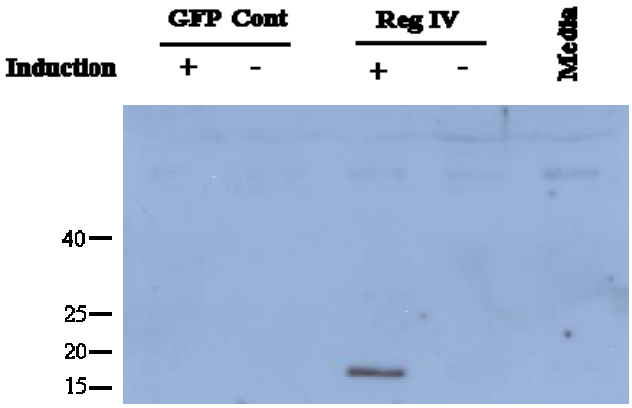
Task 1: Polyclonal anti-RegIV antibody production

As previously reported, polyclonal rabbit antisera was produced by Harlan Bioproducts (Indianapolis, IN) using synthetic peptides, RSWSGKSMGGNKHC (corresponding to residues 94–107), with a native C-terminal cysteine and TIAEYISGYQRSQPC (residues 56–69), containing a non-native C-terminal cysteine were synthesized, and coupled to maleimide-KLH. In year two, we focused on the purification of the antibody. To affinity purify anti-RegIV polyclonal antibody, antigenic peptides were covalently attached to Sulfolink-coupling gel via cysteine residue in a column (Pierce, IL). Antisera were then run through the gel column. Antibodies were eluted from the affinity column. Figure 2 shows that RegIV protein can be detected by affinity purified anti-RegIV antibodies.

Task 2: Development of monoclonal anti-RegIV

For the antigen, we had proposed to produce RegIV-GST fusion protein, but were unsuccessful. GST-RegIV fusion protein expressed in bacteria is insoluble due to the high contents of cysteine in the sequence. We then expressed RegIV as a myc-His fusion protein in Drosophila S2 cells (Initrogen, CA). We are able to express and detect the secreted Reg IV-myc-His fusion protein in S2 cell culture media. In order to develop the mouse monoclonal antibody, we used RegIV-myc-his fusion protein to immunize Balb/c mice and measured their anti-RegIV activity. The antibody titer was too low to proceed to the next step-fusion procedure. Once we obtain results from the polyclonal antibody, we will use the same KLH-peptides to immunize mice to make the monoclonal antibody.

Expression Recombinant Reg IV Protein in Drosophila S2 cells



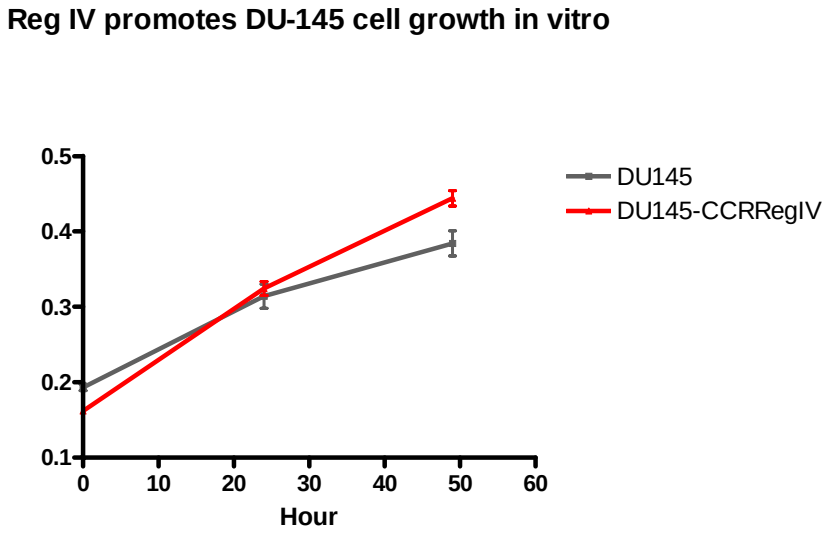
Task 3: Immunohistochemical staining and tissue arrays
The tissue array has been constructed by our collaborator. Using the affinity purified anti-RegIV antibody, we are able to detect a strong signal in LAPC-9 AI (positive control) and no signal in LAPC-9 AD (negative control, Fig. 3). We also detected strong signal in a prostate cancer sample (Fig 4). Further testing is being done to address the nuclear staining that was observed in the cancer sample. Once the background has been resolved, we will stain the array samples.

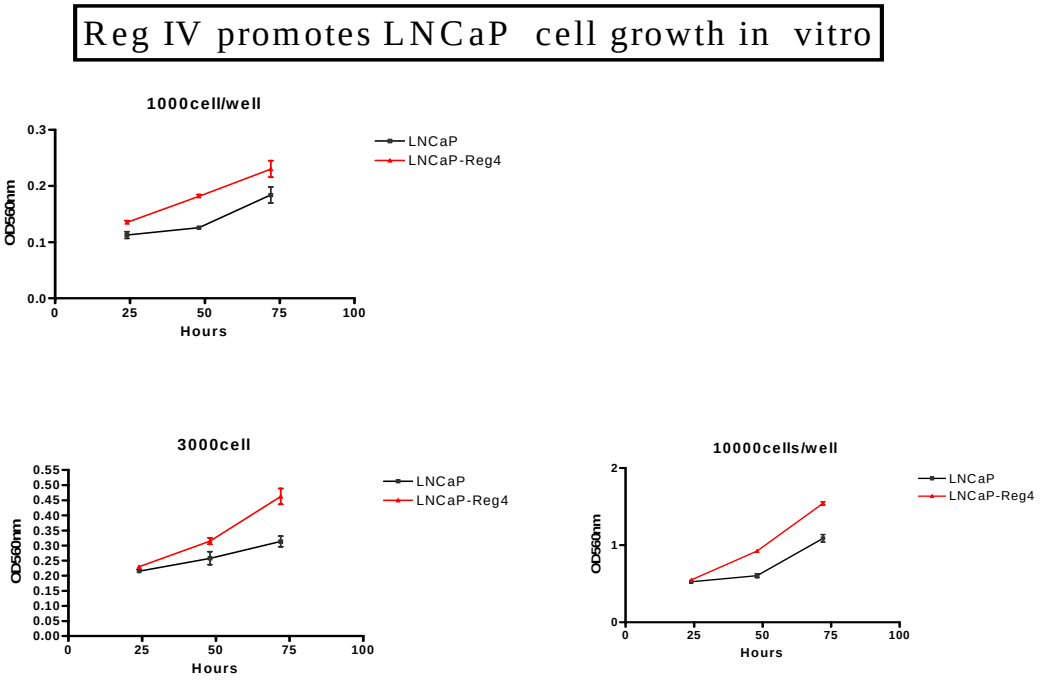
Aim 2: To detect RegIV by Enzyme-linked Immunoassay (ELISA) in sera of patients with primary and metastatic prostate cancer

Task: ELISA development
Affinity purified polyclonal anti-RegIV was biotin labeled and antibody coating conditions were tested. When the plates were tested using the RegIV conditioned media, there was very high background.

Aim 3: To determine if Reg IV plays a role in prostate cancer progression or resistance to therapy

Task 1: *In vitro* studies
LNCaP cells were stably transfected with pCDNA-REG IV expression vector or pCDNA control vector. The cells were grown on 96 well plates and measured for growth rate by MTT. It shows clearly that LNCaP-Reg IV cells grow faster than LNCaP control cells. We also observed growth advantage in DU145-RegIV cells (transduced by CCR-RegIV lentivirus) over DU145 control cells. We observed no growth differences between PC3-Reg IV and PC3 control.

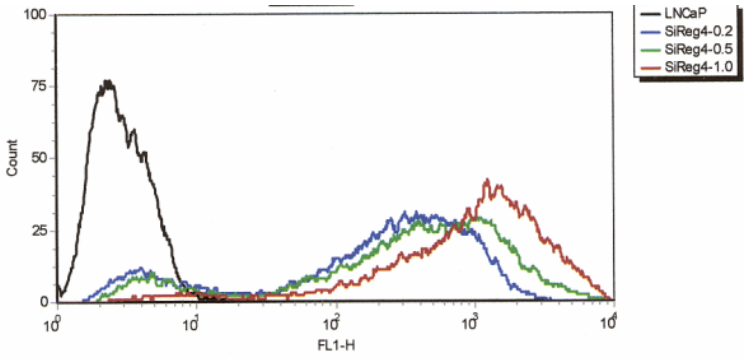


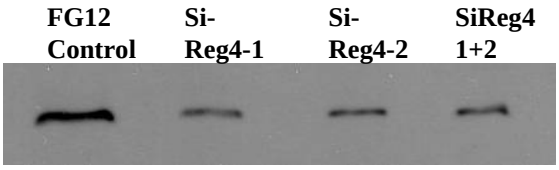


Task 2: Knockdown of RegIV

We constructed two si-RegIV lentiviral vectors to knock down RegIV RNA and protein expression. These vectors only target RegIV (not other members of Reg family). Both constructs successfully down-regulated RegIV gene expression efficiently. The constructs will be used to transduce LAPC9 cells for use in an *in vivo* competition assay to analyze the influence of RegIV gene expression in tumor growth.

Lentiviral RegIV SiRNA Reduce Reg IV Protein Expression





Cells: LNCaP-PcDNA-RegIV.His.Myc

SiReg 4-1: CTTCAGGAAGCTGAGGAAC**.**

SiReg 4-2: AAGTGGCTATCAGAGAAGC**.**

KEY RESEARCH ACCOMPLISHMENTS

- Affinity purify a polyclonal anti-RegIV antibody
- Able to express and detect the RegIV fusion protein in cell culture media
- Able to detect strong signal in immunohistochemistry control samples using the affinity purified polyclonal anti-RegIV antibody.
- Observed growth advantage in two prostate cancer lines transduced with RegIV.
- Down regulated RegIV gene expression using si-RegIV.

REPORTABLE OUTCOMES

None

CONCLUSION

We have developed and purified a polyclonal anti-RegIV that has successfully been tested by immunohistochemistry on positive and negative controls; however, nuclear staining was observed in the prostate cancer sample. We have encountered difficulties in developing a monoclonal anti-RegIV antibody due to the insolubility of the fusion protein and its high cysteine content. We will attempt to use the same KLH-peptides to immunize mice as was used for the polyclonal anti-RegIV. Once the nuclear staining background has been worked out on the cancer samples, the anti-RegIV polyclonal will be used to stain the prostate tissue array. *In vitro* studies show cells overexpressing RegIV have a growth advantage and we have successfully made two si-RegIV constructs to knock down RegIV RNA and protein expression. *In vivo* competition assays to analyze the influence of RegIV gene expression in tumor growth is currently in progress.

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