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TITLE: The Thoc1 Ribonucleoprotein as a Novel Biomarker for Prostate Cancer Treatment Assignment

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<b>14. ABSTRACT</b> Active surveillance (AS) is an option for men with low risk prostate cancer in order to reduce over treatment, but few men choose it because current prognostic indicators are imperfect. The objectives of this research are to test whether pThoc1 can improve the assignment of prostate cancer patients to therapy. We have made significant progress on the goals articulated in the Statement of Work. IRB/HRPO approval has been obtained for construction and use of new TMAs (PI Mohler and Goodrich). The TMAs from PCaP have been obtained (PI Mohler and Goodrich). Pathology analysis of 1146 patient specimens is complete and construction of TMAs initiated (PI Mohler). Optimization of TMA staining is complete and staining of TMAs initiated (PI Goodrich). IRB/HRPO approval for active surveillance specimens has been obtained (PI Mohler, Goodrich). Enrollment of prostate cancer patients on active surveillance is ongoing (PI Mohler). ELISA assays for measuring pThoc1 and pThoc1 autoantibodies have been successfully developed (PI Goodrich). Analysis of serum samples from a mouse model of prostate cancer has been performed, establishing feasibility (PI Goodrich). IRB/HRPO approval for serum samples has been obtained (PI Mohler, Goodrich). All preparative, optimization, and regulatory approval work has thus been completed, setting the stage for data gathering in year 2 of the grant. Over treatment is complicates the clinical management of prostate cancer. Improving the ability to distinguish aggressive from indolent disease is recognized as an unmet need by the 2013 PCRP Overarching Challenges. Identifying pThoc1 as a biomarker that can help meet this need will have significant impact.						
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## 1. Introduction

Active surveillance (AS) has been proposed as an option for men with low risk prostate cancer in order to reduce over treatment. Only a fraction of eligible men choose AS, however, because current prognostic indicators are imperfect. Biomarkers that improve upon PSA levels, clinical stage and Gleason score to distinguish between prostate cancers that can be observed safely from those that require immediate treatment could help “right size” recommended treatment. The objectives of this proposal are to test whether pThoc1 can improve the assignment of prostate cancer patients to therapy, to test whether pThoc1 correlates with observed racial disparities in prostate cancer mortality, to determine whether pThoc1 can identify active surveillance patients whose prostate cancer will progress, and to develop methods to quantitate pThoc1 or pThoc1 autoantibody in serum. The general study design is to assay pThoc1 in independent cohorts of clinically annotated prostate cancer biospecimens for which clinical and follow up data is available using previously developed antibody reagents and immunostaining methods. Over treatment is a critical issue complicating the clinical management of prostate cancer. Improving the ability to distinguish aggressive from indolent disease in men newly diagnosed with prostate cancer is recognized as an unmet need by the 2013 PCRP Overarching Challenges. Identifying pThoc1 as a biomarker that can help meet this need will have significant impact.

## 2. Keywords

Prostate cancer, biomarker, active surveillance, prognostic indicator, tissue microarray, immunostaining, ribonucleoprotein

## 3. Accomplishments

There are three major goals for the proposed work: 1) Characterize pThoc1 levels in independent cohorts of human prostate cancer radical prostatectomy specimens. 2) Characterize pThoc1 levels in a cohort of human prostate cancer patients on active surveillance. 3) Test whether pThoc1 or autoantibodies against pThoc1 can be detected in the serum of prostate cancer patients.

The major accomplishments achieved during the first funding period of the grant include successfully securing all IRB/HRPO regulatory approvals to conduct the work, pathological assessment of 1146 patient specimens for use in constructing tissue microarrays, developing ELISA assays to detect pThoc1 and pThoc1 autoantibodies, and validating those assays using a mouse model of prostate cancer. With respect to the approved Statement of Work, the following objectives were accomplished: IRB/HRPO approval has been obtained for construction and use of new prostate cancer TMAs (PI Mohler and Goodrich). The TMAs from PCaP have been obtained (PI Mohler and Goodrich). Pathology analysis of 1146 patient specimens is complete and construction of TMAs initiated (PI Mohler). Optimization of TMA staining is complete and staining of TMAs initiated (PI Goodrich). An example of PCaP TMA staining is provided in figure 1. IRB/HRPO approval for active surveillance specimens has been obtained (PI Mohler, Goodrich). Enrollment of prostate cancer patients on active surveillance is ongoing (PI Mohler).

ELISA assays for measuring pThoc1 and pThoc1 autoantibodies have been successfully developed (PI Goodrich). A sandwich ELISA assay has been developed for detecting Thoc1 protein in serum samples. Recombinant Thoc1 protein was produced in E.coli and purified by affinity chromatography. This control protein was used to develop the sandwich ELISA assay, and a standard curve using the assay is shown in figure 2. An indirect ELISA assay has been developed for detecting autoantibodies against Thoc1 protein in serum samples. Purified anti-Thoc1 mouse monoclonal antibody was used as control antibody for generating a standard curve (figure 3). Specificity of the assay was tested using alternative anti-Thoc1 antibody or an irrelevant negative control antibody (figure 4).

IRB/HRPO approval for human serum samples has been obtained (PI Mohler, Goodrich). Task 1, specific aim 1 is nearly complete. Task 1, specific aim2 is nearly complete. Task 1, specific aim 3 is complete. Task 2, specific aim 3 is 30% complete. Overall, optimization, validation, and regulatory approval work has been completed setting the stage for data gathering for all specific aims during the second funding period of the grant.

The grant does not support training and professional development, so there is nothing to report.

The work is at a preliminary stage, so results have yet to be disseminated through publication or presentations.

Year 2 of the grant will focus on data gathering and initial data analysis on all specific aims. With respect to the approved Statement of Work, Task 2 of specific aim 1 will be completed (immunostaining prostate TMAs, PI Goodrich). Task 3, specific aim 1 will be initiated (scoring TMAs and correlating with clinical data, PI Mohler and Goodrich). Task 1, specific aim 2 will be completed (immunostaining active surveillance specimens, PI Goodrich). Task 2, specific aim 3 will be initiated (assay serum for pThoc1 and pThoc1 autoantibody, PI Goodrich).

#### **4. Impact**

What was the impact on the development of the principal discipline(s) of the project? ELISA assays were developed for measuring pThoc1 or pThoc1 autoantibodies in serum. Such assays are not currently available, so these assays will permit measurement of this ribonucleoprotein and autoantibodies directed against it. By correlating these measurement against clinical data, we will determine whether pThoc1 or pThoc1 autoantibodies are a useful serum biomarker for prostate cancer. If so, the development of these assays will impact the diagnosis of prostate cancer and possibly other cancers.

If pThoc1 or pThoc1 autoantibodies prove to be a useful serum biomarker for prostate cancer, the work may impact development of diagnostic/prognostic products based on pThoc1.

The presence of the THO ribonucleoprotein complex, or antibodies directed against it, have not been measured in serum or other bodily fluids, either in humans or animal models. These measurements may impact understanding of ribonucleoproteins in general and their ability to elicit autoantibody responses.

#### **5. Changes/Problems**

Nothing to report.

#### **6. Products**

As the work is still at a preliminary stage, it has not yet been disseminated through publications, conference papers, website(s), or presentations. No inventions, patent applications, and/or licenses have been produced.

ELISA assays were developed for measuring pThoc1 or pThoc1 autoantibodies in serum. If utility of these assays as serum biomarkers of prostate cancer aggressiveness is established, results will be shared through journal publications and meeting presentations.

#### **7. Participants & Other Collaborating Organizations**

David W. Goodrich, Ph.D. (Initiating PI): No change.

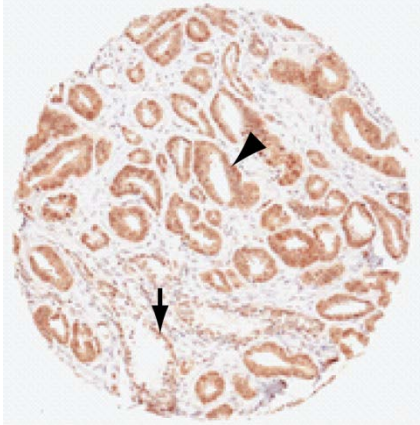
Meenalakshmi Chinnam, Ph.D. (postdoctoral fellow): No change.

There has been no change in active other support for the PI or postdoctoral fellow since the original grant submission. No other organizations are involved in the research.

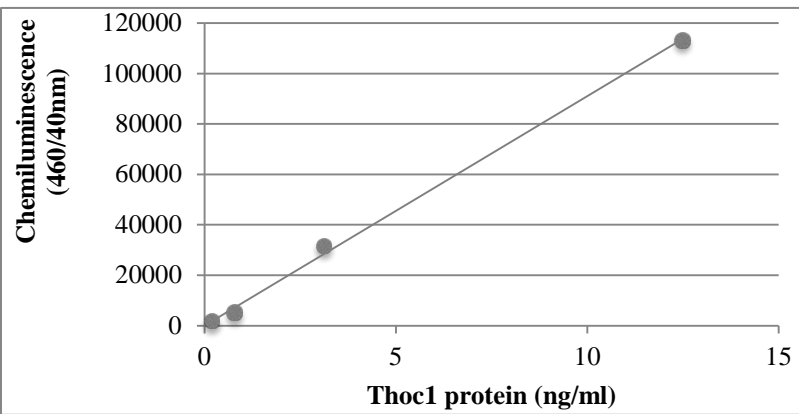
#### **8. Special Reporting Requirements**

This grant funds a Synergistic Idea Development Award in collaboration with Dr. James Mohler (Partnering PI, Roswell Park Cancer Institute). Dr. Mohler will be submitting an independent annual report describing his aspect of the work.

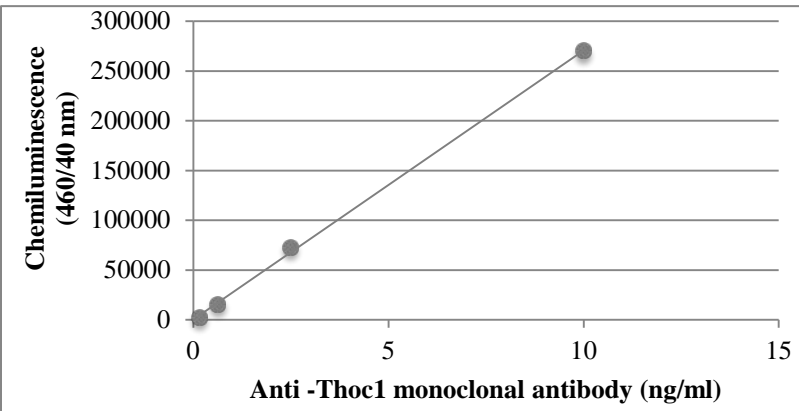
9. Appendices



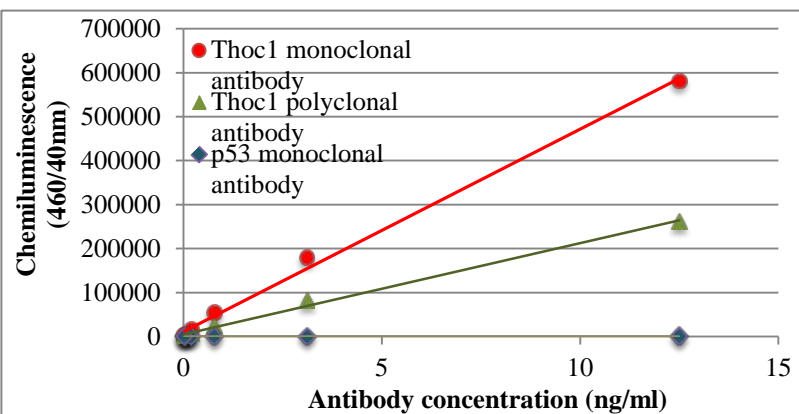
**Fig 1. Thoc1 immunostaining of prostate cancer tissue from PCaP TMA.** TMAs from PCaP project were obtained and immunostained for Thoc1 protein using our optimized protocol. Increased Thoc1 expression is observed in tumor ducts (arrowhead) compared to cells in an adjacent benign duct (arrow).



**Fig 2: Measurement of Thoc1 protein by sandwich ELISA.** A range of diluted, purified Thoc1 control protein (0.2-12.5 ng/ml) was assayed by ELISA and the chemiluminescence (460/40nm) recorded after subtracting assay blank. The lowest amount of Thoc1 protein detectable is 200 pg/ml or 10 pg/well.



**Fig 3: Measurement of anti-Thoc1 antibody by indirect ELISA assay.** A standard curve was generated a dilution series (0.16-10 ng/ml) of purified anti-Thoc1 control antibody. The lowest amount of anti-Thoc1 antibody detectable is 160 pg/ml or 8 pg/well.



**Fig 4: Specificity of indirect ELISA assay for detecting anti-Thoc1 antibody.** Standard curves were generated with two different anti-Thoc1 antibodies and a p53 specific, negative control antibody. The negative control antibody did not generate signal above background, demonstrating specificity.