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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT 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 is complete (PI Mohler). Immunostaining of all TMAs is complete (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). Immunostaining of prostate cancer biopsy specimen of patients who qualified for active surveillance is complete (PI Goodrich). ELISA assay development for measuring pThoc1 and pThoc1 autoantibodies is complete (PI Goodrich). IRB/HRPO approval for serum samples has been obtained (PI Mohler, Goodrich). Analysis of serum samples from prostate cancer patients of PCaP cohort is complete (PI Goodrich). Analysis of serum samples from prostate cancer patients and corresponding normal subjects of RPCI cohort has been initiated (PI Goodrich). Year 4 of the grant will finish data acquisition and data analysis. Over treatment 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.

15. SUBJECT TERMS
Prostate cancer, biomarker, active surveillance, prognostic indicator, tissue microarray, immunostaining, ribonucleoprotein

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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 past three years of 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 and optimizing ELISA assays to detect Thoc1 protein and anti-Thoc1 autoantibodies, and validating those assays using human serum from normal healthy subjects. With respect to the approved Statement of Work, the following objectives were accomplished: Construction of new prostate cancer TMAs (PI Mohler and Goodrich) of 1146 patient specimens is complete and immunostaining of these TMAs for Thoc1 protein (figure 1) is complete (PI Goodrich). Pathological scoring and data analysis of the TMAs is underway (PI Goodrich and Mohler). The TMAs from PCaP project have been obtained (PI Mohler and Goodrich) and immunostaining of TMAs for Thoc1 protein (figure 2) is complete (PI Goodrich). Pathological scoring and data analysis of the TMAs is underway (PI Goodrich and Mohler). Enrollment of prostate cancer patients on active surveillance is ongoing (PI Mohler). Thoc1 immunostaining of prostate biopsy tissue of patients from PCaP and RPCI cohort who are candidates for active surveillance is complete (figure 3 and 4 respectively). Pathological scoring and data analysis of the immunostained biopsy specimen is underway (PI Goodrich and Mohler). Analysis of serum samples of PCaP cohort for Thoc1 autoantibody levels is complete (figure 7). Analysis of serum samples of prostate cancer patients from RPCI cohort and serum samples from age and race matched normal healthy male subjects obtained from RPCI-DBBR for Thoc1 autoantibodies is underway (PI Goodrich).

A three gene panel constituting PMP22, CDKN1A and FGFR1 has been shown to stratify low gleason score prostate cancers into indolent and aggressive cancer (Irshad et al., 2013). This panel of genes has been shown to have increased expression in low Gleason score prostate cancers that are likely to remain indolent compared to those which will progress and become aggressive prostate cancer. Thoc1 protein on the other hand is highly expressed in those low Gleason score prostate cancers that are likely to progress to aggressive prostate cancer (Chinnam et al., 2014). Hence, Thoc1 expression in low Gleason score prostate cancer is complimentary to that of the panel of PMP22, CDKN1A and FGFR1. Addition of this complimentary panel of genes to our analysis will help us robustly determine if Thoc1 can stratify low Gleason score prostate cancers into indolent and aggressive prostate cancer. Immunostaining for PMP22, one of the complimentary antigen to Thoc1, has been optimized (PI Goodrich). A set of TMAs made from 700 patients available at RPCI has been immunostained for PMP22 (figure 5).

ELISA assays for measuring pThoc1 and pThoc1 autoantibodies have been successfully developed (PI Goodrich). A competitive ELISA assay has been developed and validated for detecting Thoc1 autoantibodies (figure 6). Thoc1

autoantibody levels were measured in serum samples of prostate cancer patients from PCaP cohort using competitive ELISA (figure 7). A limited number of serum samples from healthy male donors were analyzed for Thoc1 autoantibody levels (figure 7). 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 8. Measurement of serum Thoc1 protein concentration of PCaP cohort samples is ongoing.

IRB/HRPO approval for human serum samples has been obtained (PI Mohler, Goodrich). Task 1 and 2, specific aim 1 is complete. Task 1 and 2, specific aim2 is complete. Task 1, specific aim 3 is complete. Task Task 2, specific aim 3 is ongoing. Overall, optimization, validation, and regulatory approval work has been completed and data gathering for all specific aims is ongoing.

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

Data collection and analyses are in progress, so results have yet to be disseminated through publication or presentations.

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 measurements 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 ribonucleoprotin 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 ongoing, 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
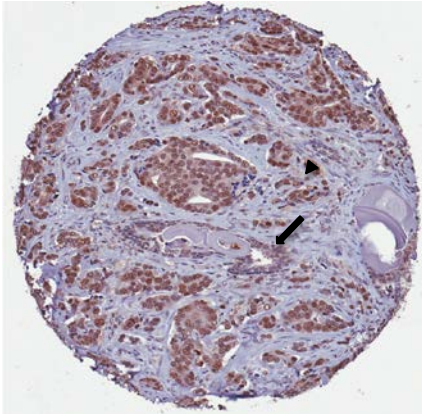
Fig 1. Thoc1 immunostaining of prostate cancer tissue from newly constructed TMA. Newly constructed prostate cancer TMAs were immunostained for Thoc1 protein using our optimized protocol. Increased Thoc1 expression is observed in cancer lesions (arrowhead) compared to cells in an adjacent benign gland (arrow).
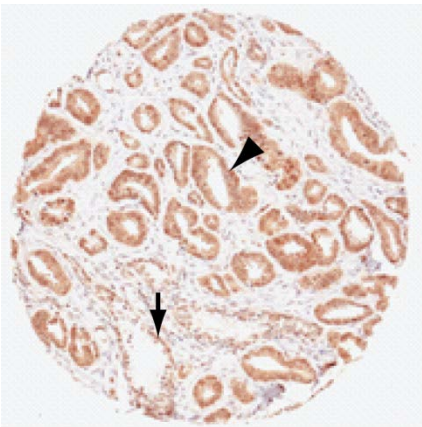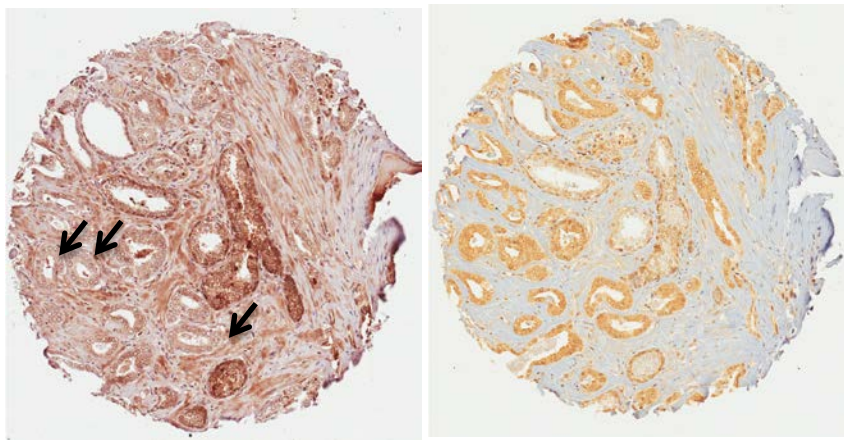

Fig 2. Thoc1 immunostaining of prostate cancer tissue from PCaP TMA. TMAs from PCaP project were immunostained for Thoc1 protein using our optimized protocol. Increased Thoc1 expression is observed in cancer glands (arrowhead) compared to cells in an adjacent benign gland (arrow).
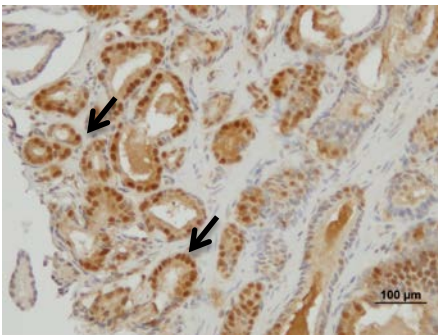
Fig 3: Thoc1 immunostaining of prostate biopsy tissue from PCaP. Biopsy specimen of patients in PCaP cohort who qualified for active surveillance were immunostained for Thoc1 protein. Increased Thoc1 expression is observed in atypical cells of cancerous glands (arrows) compared to adjacent benign glands. Also, the staining pattern is heterogenous.
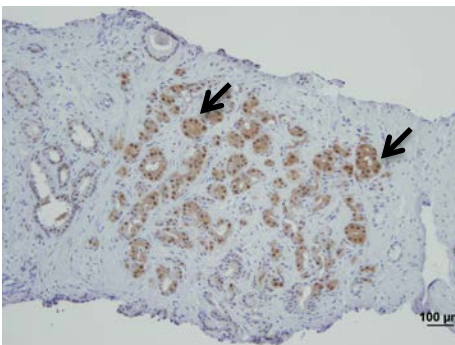

Fig 4: Thoc1 immunostaining of prostate biopsy tissue from RPCI cohort. Biopsy specimen of patients in RPCI cohort who qualified for active surveillance were immunostained for Thoc1 protein. Increased Thoc1 expression is observed in atypical cells of cancerous glands (arrows) compared to adjacent benign gland. Also, the staining pattern is heterogenous.

PMP22

Thoc1

Fig 5: PMP22 immunostaining of prostate cancer tissue from RPCI TMA. A set of prostate cancer TMAs from RPCI cohort was immunostained for PMP22 protein (shown on left). TMAs from the same cohort were previously analyzed for Thoc1 protein (shown on right). Note decreased PMP22 staining in cancer ducts (arrows) whereas the same cancer ducts on adjacent TMA show increased Thoc1 expression.
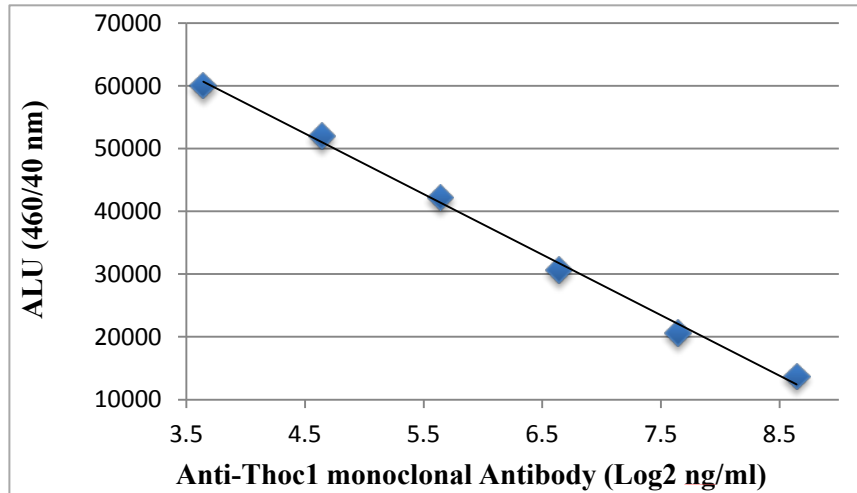
Fig 6: Measurement of anti-Thoc1 antibody by competitive ELISA assay. A competition standard curve was generated from a dilution series (12.5-400 ng/ml) of purified anti-Thoc1 control antibody in competition with anti-Thoc1 biotinylated antibody.
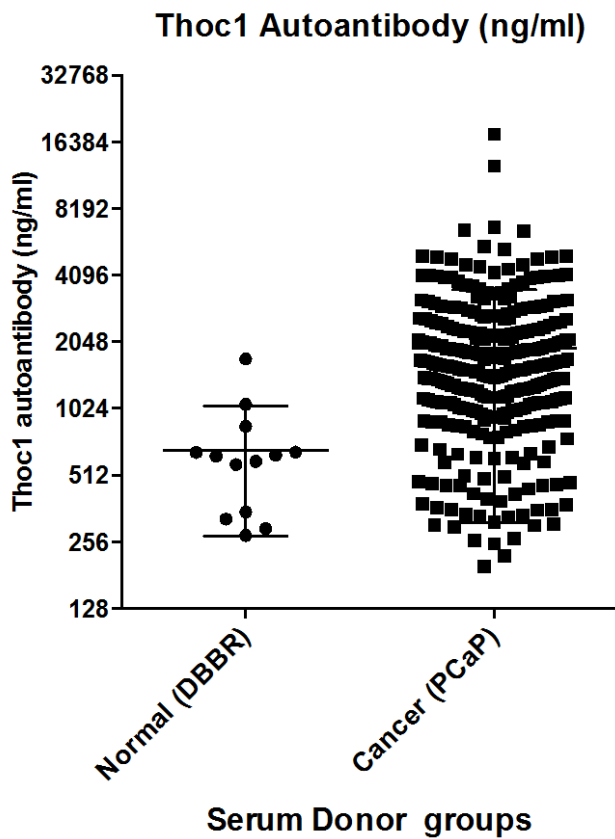
Fig 7. Serum Thoc1 autoantibody levels of prostate cancer patients (PCaP): Serum Thoc1 autoantibody levels of 325 prostate cancer patients in PCaP cohort were measured by competitive ELISA assay. Thoc1 autoantibody levels vary considerably. For comparison a limited number of serum samples of normal healthy males were obtained from RPCI-DBBR and Thoc1 autoantibody levels measured. Thoc1 autoantibody levels in normal subjects varied to a lesser extent compared to prostate cancer patients in PCaP cohort. The average serum Thoc1 autoantibody concentration was significantly higher in prostate cancer patients compared normal healthy male donors (student's t test, p=0.0034).

10. References:

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Chinnam M, Wang Y, Zhang X, Gold DL, Khoury T, Nikitin AY, Foster BA, Li Y, Bshara W, Morrison CD, Payne Ondracek RD, Mohler JL, Goodrich DW.
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