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Prostate Specific or Enriched Genes as Composite Biomarkers for Prostate Cancer

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**14. ABSTRACT**
Purpose and scope of research: To evaluate prostate specific genes such as WDR19, NDRG1, TAGLN2 as diagnosis and prognosis markers for prostate cancers. Major findings: (1) We have determined that WDR19, NDRG1 are not as good a marker as PSA for prostate cancer stratification. (2) We developed a mouse antibody for a putative prostate biomarker TAGLN2. (3) We have generated a novel panel of more than 10 prostate-specific proteins that expressed highly in prostate tissues. We will develop multiplexed antibody assays as well as mass spectrometry based targeted proteomics approach for validating these candidate biomarkers in patient serum samples.

**15. SUBJECT TERMS**
prostate cancer, biomarker, ELISA assay, MRM, targeted proteomics, Mass Spectrometry (MS).

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INTRODUCTION:

The long-term goal of this proposal is to establish a panel of multiple protein biomarkers for prostate cancer diagnosis and stratification. The originally proposed aims were to develop and optimize sandwich ELISA assays for WDR19, NDRG1, or other novel prostate-specific biomarker candidates. During the past two years, we have evaluated the utility of WDR19 and NDRG1 as prostate cancer biomarkers by screening a large cohort of prostate cancer serum samples, and determined that none of them is as good a marker as PSA. This report summarizes the progress that we have made during the last year, and our adjusted efforts toward validating new prostate-specific protein biomarkers.

BODY:

Development of monoclonal antibody for TAGLN2

We have demonstrated previously that TAGLN2 is a putative biomarker under-expressed in prostate cancer patient serum. We had a polyclonal anti-rabbit antibody against TAGLN2. To develop sandwich ELISA assay for TAGLN2, we need another antibody to pair with the anti-rabbit antibody. Initially, we ordered two custom antibodies from Covance Inc: one anti-rat polyclonal antibody and one anti-mouse monoclonal antibody against full-length TAGLN2. We have received the anti-rat antibody but the anti-mouse antibody generation failed. We then contracted another company (AbMax) to generate two monoclonal antibodies, one against the N-terminal portion (ISB101) and the other one against the C-terminal portion of the protein (ISB102). After cell fusion, AbMax conducted ELISA screening to identify the hybridomas, which carry the antibodies.

For ISB102, AbMax succeeded at the 1st cell fusion. They identified 6 hybridomas, tested their specificity against the C-terminal portion of the protein using ELISA and Western Blot. They sent us 6 supernatants for further characterization. We tested, via Western Blot, whether the supernatants can recognize 1) recombinant full length TAGLN2, 2) TAGLN2 in prostate cancer cell lysates, and 3) TAGLN2 in human prostate cancer serum. Five of the supernatants at dilution 1:4 recognize the α form of TAGLN2 in LNCaP cell lysates but not in human serum. We picked three of the supernatants to test their specificity again in human serum on Western Blot using 1:1 dilution. Only one of them seem recognize the β form of TAGLN2 in human prostate cancer serum. Since the band on the Western blot is extremely faint, AbMax produced a small amount of purified antibody from ascites. We tested the purified antibody in human prostate cancer serum using Western Blot. However, the purified antibody did not produce a positive signal on the Western Blot. Since the antibody can recognize the recombinant full length protein, we are testing whether this ISB102 antibody can pair with our polyclonal anti-rabbit antibody on sandwich ELISA. For ISB101, no viable antibody was produced. AbMax is working on generating a monoclonal antibody against the full length TAGLN2 as a remedy.

In the second half of the last funding period, while waiting for the TAGLN2 antibodies, we renewed a search for additional prostate-specific biomarkers. During this period, the Hood laboratory developed a sophisticated software tool for data mining, so that tissue-specific information can be extracted from both public and ISB’s in house databases. Through this more extensive bioinformatics analysis, we compiled a new list of prostate-specific proteins. One remarkable feature of this new panel of proteins is that, their transcript levels are enriched at least 10 fold in prostate tissue comparing to any other tissues examined (figure 1). Thus, in essence, they represent true prostate-specific markers.
Figure 1. Prostate-specific gene expression profiles of a panel of 15 genes across a panel of 28 different human tissues. Data was generated using Genecards tools from three public datasets: GeneNote, GNF normal, and GNF tumor. The highest expression was observed in prostate tissue (blue color).

A daunting challenge we encountered during the past two years to develop efficient ELISA assays for target proteins is the availability of good antibody pairs. We have been actively seeking alternative technological platforms for validating new protein markers in patient sera. The most promising alternative by far is mass spectrometry (MS) based targeted proteomics approach dubbed MRM for Multiple Reaction Monitoring. After extensive experimentation on various MS instruments and optimization of front-end serum enrichment methods, it appears that we finally arrive at a stage where peptides derived from multiple proteins can be quantitatively assayed simultaneously in a single MS run. With the extension for one more year, we will be able to test these promising new biomarkers for their utilities in prostate cancer patient sera.
KEY RESEARCH ACCOMPLISHMENTS:

- Developed a mouse antibody for TGNL2.
- Established a novel panel of prostate-specific proteins to be tested by both antibody and MS based assays.

REPORTABLE OUTCOMES:

A panel of prostate-specific genes with the highest expression in prostate tissues.

CONCLUSION AND FUTURE PLANS:

Better markers than PSA are needed. These markers in combination with others such as PSA may turn out to provide a very good assay for prostate cancer. It is clear that any single marker—or perhaps any combination of two markers may not themselves be good markers. However, 4-6 such markers might constitute a powerful test for prostate cancer.

While antibody-based ELISA assays are gold standard for protein bioassays, lack of matching antibody pairs for any given protein targets drastically hampered the transition from biomarker discovery to clinical validation. MS based targeted proteomic strategies provide a viable alternative for quantitative detection of multiple protein biomarker candidates at considerable sensitivity and throughput. We will continue to explore both approaches in validating our new collection of prostate-specific protein panel described above in prostate cancer patient sera.

Most of these proteins have antibodies available. We will explore a new strategy for multiplex protein measurement based on proximity ligation assay, which in theory requires only one polyclonal antibody. We will link the antibodies with unique oligonucleotides. Binding of the antibodies to their targets brings these oligonucleotide tails into proximity. Ligating the tails with a connector oligonucleotide will form a unique amplicon surrogate for the target protein, which can be quantitatively detected by real-time PCR. By choosing different combination of oligonucleotide and antibodies, multiplexing can be accomplished. We will choose 10 candidate proteins for multiplex measurement, and compare the data with PSA alone.

For targeted MS-based approaches, we will choose 3 peptides from each protein, obtain heavy isotopic labeled peptides from commercial source. These heavy labeled peptides will be spiked into serum samples and measure by MRM-MS for relative expression level of the corresponding proteins. We plan to further test 10 prostate-specific markers through MRM-MS in prostate patient samples.

REFERENCE:

None.

APPENDICE:

None.