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14. ABSTRACT Metastatic prostate cancers are commonly treated by agents designed to suppress androgen receptor (AR) signaling mediated by the full-length AR (AR-FL). Why some patients progress rapidly after treatment while others benefit with prolonged remission is an unsolved question. We propose approaches to develop molecular indicators of response and resistance that will enable prediction (before therapy) or early detection (during therapy) of therapeutic benefit. We will test the hypothesis that AR splice variants (AR-Vs) are molecular indicators of castration-resistant prostate cancer (CRPC). During this funding period, we achieved a major milestone by completing experimental phase of the proposed transcriptome sequencing studies, and began the data analysis phase of the project. In addition, we have identified and acquired biopsy specimens that will be required for the proposed in-situ detection methods. We conclude that androgen receptor splice variants can be detected by RNA sequencing in clinical specimens derived from patients with castration-resistant prostate cancer.					
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Introduction

Hormone therapies block androgen production and/or androgen receptor (AR) function leading to a period of clinical regression varying from months to more than 6 years among patients treated for metastatic prostate cancer. Because restored AR signaling is the key determinant of castration-resistant prostate cancer (CRPC), aberrant variants of the androgen receptor may mediate and indicate therapeutic resistance. Our recent efforts (1-3) have established that truncated androgen receptor splice variants (AR-Vs) are resistant to castration therapies that target the canonical full-length AR (AR-FL). We are now equipped with the knowledge that AR-Vs are both structurally and functionally distinguishable from the full-length AR (AR-FL), which remains the key target in prostate cancer drug design. We view these new discoveries and knowledge as opportunities that have yet to be capitalized to address the unmet need of developing indicators of castration resistance. In our previous studies, we have shown elevated expression of the AR-Vs following suppression of AR-FL (3). The primary purpose of the project is to discover and develop molecular indicators of CRPC by targeting the AR-Vs. We will test the hypothesis that AR-Vs are molecular indicators of CRPC. The scope of the proposed research is: 1) to perform RNA-Seq targeting transcripts originated from the human AR gene locus for *ab initio* cataloging of AR-Vs that define CRPC; and 2) to establish the proof-of-principle that AR-Vs detected prior to the initiation of hormone therapies may be used to predict CRPC progression.

Body

Findings resulting from Task 1: To employ a novel targeted RNA-Seq method for *ab initio* cataloging of androgen receptor variants that define CRPC (Months 1-24).

Summary: By generating raw sequencing data using RNA-seq from 48 clinical specimens, we have achieved a critical project milestones associated with Task 1 as outlined in SOW. As stated in SOW, data analysis involving all specimens will be performed during the second year of the funding period. On the basis of initial findings from three specimens, we conclude that AR-V7, one of the most important androgen receptor splice variant (4), is abundantly expressed in CRPC and detected by RNA-seq.

Supporting data: Quantitative RT-PCR results suggest that the expression level of each individual AR-V is often a small fraction of the total level for AR-FL. For example, AR-V7 represents only about 1% to 5% of the total AR in the vast majority of CRPC specimens examined (1, 5). Because of the lower abundance of AR-V than AR-FL, the clinical significance and functional role of AR-V has been debated (4-6). However, we posit that 1) accurate quantification of AR-V transcripts would be compromised by unknown splice junctions as well as various technical constraints of the conventional RT-PCR methodology and 2) even at a small fraction of the AR-FL, AR-V expression in treatment-resistant specimens may reach levels equivalent to those of AR-FL in untreated tumors because AR-FL is often massively overexpressed in CRPC (7), thus conferring

functional significance when AR-FL is suppressed. To address accurate quantification, we performed RNA-seq in three independent CRPC specimens using the Illumina Hi-Seq 2000 platform (Figure 1). Comparing to qRT-PCR data from the same specimens, we found that RT-PCR *underestimated* the AR-V7 abundance by an average of 5 fold, and that in one CRPC specimen the ratio of AR-V7 to AR-FL reached 41%, using the number of AR-FL- and AR-V7-specific exon-spanning sequence reads as proxies for relative transcript abundance (Figure 1). Taken together, these data suggest that AR-V7 is a high-abundance transcript in a subset of clinical CRPC, and may be readily detected in CRPC cells. Other known AR-Vs are either orders of magnitude lower in abundance than AR-V7, or functionally inactive (2). Another constitutively active AR-V, ARV567ES, is frequently expressed in CRPC (8). Interestingly, splice junctions specific for ARV567ES are not detected by the three RNA samples sequenced so far (Figure 1). These findings suggest that although other variants may contribute to CRPC, AR-V7 is the best candidate for designing detection assays to be used in clinical specimens.

Findings resulting from Task 2: To establish the proof-of-principle that AR-Vs detected prior to the initiation of hormone therapies may be used to predict CRPC progression (Months 1-36).

According to our project plan in SOW we will focus on sample preparation during year 1 of the project. Experimental data and findings will not be available until later years of the project period.

Key Research Accomplishments

1. Completed raw RNA-seq data collection from 48 samples.
2. Established that RNA-seq can be used to reliably estimate the abundance of AR variant transcripts.

Reportable Outcomes

Manuscripts: None at this time.

Presentations: None at this time.

Grant Applications:

Title: The aberrant androgen receptor underlies abiraterone/enzalutamide resistance

Supporting Agency: NIH (Luo - PI)

Name of Procuring Contracting/Grants Officer: TBD - NIH

Address of Funding Agency: 9000 Rockville Pike, Bethesda, MD 20892

Performance Period: 4/1/2014 - 3/31/2019

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Conclusion

We conclude that androgen receptor splice variants can be detected by RNA sequencing in clinical specimens derived from patients with castration-resistant prostate cancer. Our findings suggest that previous methods of detection may have *underestimated* the AR splice variant transcript abundance. The implication is that AR splice variants may be more abundant than previously thought, allowing robust detection in clinical specimens.

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Appendices

None

Supporting Data (Figure 1 and legend)

Figure 1. RNA-seq results illustrating the splice junction tracks for the androgen receptor locus in 3 CRPC specimens. The number of reads specific to each splice junction were compared with data derived from conventional RT-PCR. For example, the number of reads spanning AR-V7 splice junction (second blue arrow) is 41 in CRPC #2 and reads spanning AR-FL splice junction is 111, while the V7/FL ratio in this sample by QRT-PCR is 8%, suggesting QRT-PCR underestimates the true ratio of AR-V7/AR-FL by 5 fold. Blue arrows indicate splice junctions. The number of reads were extracted from 3 specimens (CRPC #1, #2, and #3).

