DNA Methylation Screening of Primary Prostate Tumors Identifies SRD5A2 and CYP11A1 as Candidate Markers for Assessing Risk of Biochemical Recurrence

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Introduction
Prostate Cancer (PCa) is the second-leading cause of cancer-related deaths among American men. Currently, the best way to determine the probability of a prostate tumor becoming aggressive after the “Intermediate” stage involves an invasive prostate biopsy procedure to observe the pathology (ie. the organization of cells) of the tumor. This procedure may produce unintended harmful consequences to the patient and may over- or under-diagnose a patient disease as well. A typical treatment for supposed “serious cases” of the disease is to remove the prostate in a procedure called radical prostatectomy (RP). Post-RP, after the prostate is removed, doctors continue to observe the patient that the disease is no longer present (NED). However, occasionally doctors detect levels of the prostate-specific antigen (PSA) which indicate the patient has experienced biochemical recurrence (BCR) and that there may be residual prostate tumor in the body. It is critical that we discover novel prognostic biomarkers to better predict a patient’s disease outcome in a less invasive manner.

PCa is dependent on the androgen biosynthesis pathway (ABP). During normal prostate development and development within the tumor (tumorigenesis), the androgen receptor (a transcription factor which turns on/off genes) is activated by dihydrotestosterone (DHT). DHT is a product of the ABP. The ABP however has been implicated in prostate cancer (PCa) progression and can be used to predict disease and drug resistance. Genome Biol. 2014;15(8):449.

Materials and Methods
DNA Methylation Quantification of Prostate Normal and Tumor Tissue and Cell-free Plasma: The first DNA methylation quantification method we used was Methyl-binding domain capture sequencing (MBDCap-seq)(Fig. 1A). MBDCap-seq identified “large” differentially methylated regions (DMRs) in primary tumors of patients who later experienced recurrent PCa (biochemical recurrence, BCR) or did not (no evidence of disease, NED), respectively. The second method we used for quantifying DNA methylation was pyrosequencing. Pyrosequencing is more specific for measuring the percentage of methylation at potential sites of DNA methylation (CpG sites). We measured DNA methylation of cfDNA samples from 86 PCa patients taken at and/or post-radical prostatectomy (RP) using univariate and multivariate prediction analyses.

RESULTS.

Putative DMRs in 13 of 30 ABP-related genes were found between two loci - SRD5A2 and CYP11A1, which also correlated with their decreased expression, in tumors with subsequent BCR development. Their aberrant cfDNA methylation was associated with detectable PSA in post-radical prostatectomy (RP) using univariate and multivariate prediction analyses.

Conclusions
By using two separate methods, we have shown that the DNA methylation of the promoter regions for some of the ABP-related genes are modified significantly between recurrent and non-recurrent PCa. Additionally, there exists a significant correlation between the promoter regions of SRD5A2 and CYP11A1 and their gene expression (data not shown), implicating an epigenetic role in the regulation of the ABP. Furthermore, the detectable changes of DNA methylation at SRD5A2 and CYP11A1 may serve as non-invasive plasma cfDNA prognostic biomarkers for BCR PCa. A broader investigation of the DNA methylation of the DNA regions beyond the promoter regions of the ABP-related genes could uncover clinically significant DMRs at other important expression regulatory regions as well.

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