Epigenetic Biomarker Identification in Prostate Cancer using Liquid Biopsy Approaches

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Background & objectives

Prostate cancer is the second leading cause of death in men affected by cancer. Once metastasis occurs, only one-third of the patients will survive for five years after diagnosis. Metastatic prostate cancer can exist as localized, oligometastatic or polymetastatic tumors. The success of the therapeutic strategies for prostate cancer largely depends on the selection of appropriate interventions based on the precisely determined tumor state. Earlier determining therapeutic approaches, longitudinal monitoring of tumor progression, and distinguishing treatment effects from relapse are primarily limited by the invasive nature of conventional tissue biopsy methods and the lack of tumor tissues. However, with the advent of minimally invasive liquid biopsy methods, prostate cancer patient management can be greatly improved. The main objective of this study is to identify epigenetic alterations of the circulating cell-free DNA (cfDNA) from the prostate cancer patient's blood. More specifically, cfDNA differentially methylated regions (DMRs) were determined to understand the common and unique epigenetic changes between primary and oligometastatic prostate cancer patients.

Methods

Methylated cfDNA fragments from oligometastatic prostate cancer patients' plasma were captured using antibody-based cf-MEDIP protocol followed by next-generation sequencing of the amplified libraries. In parallel, prostatectomy, oligometastatic lymph node, and adjacent normal prostate tissue libraries were also prepared and sequenced. Sample-wise sequencing data were analyzed to generate BAM files using a customized bioinformatic pipeline consisting of FastQC (sequencing reads quality assessment), trimgalore (adaptor trimming), bowtie2 (alignment to human reference genome hg38), and samtools (for sorting, indexing, and PCR duplicate removal) implementations. BigWig files generated using the deeptools package were used for enriched peaks visualization in Integrated Genomics Viewer. R-package QSEA was used to generate differential methylome profiles of primary and oligometastatic tumors.

Results

Higher recapture efficiency of spike-in 5mC DNA fragments as compared to unmodified C fragments shows the higher specificity of 5mC-specific antibody at an experimental level. In addition, quality checks using the QSEA package also revealed a higher enrichment efficiency

with < 20% background reads across samples. Moreover, principal component analysis based on CpG-Island promoters methylation status revealed a higher degree of heterogeneity in tumor samples as compared to the healthy control group. Comparative analysis of DMRs set of prostatectomy, oligometastatic tumor, and prostate cancer patient plasma cfDNA revealed ~1500 genomic locations of 500 window size. These DMRs encoded for 789 human genes are conserved. GO analysis revealed that most of these conserved genes are associated with signaling pathways. In addition to exploratory analysis with respect to DMRs, the development and validation of machine learning models that are able to distinguish tumor patients from healthy individuals is underway.

Conclusion

Several differentially methylated regions are conserved between primary and oligometastatic prostate cancer patients, and a subset of it can be captured on plasma cfDNA.