Non-invasive prostate cancer subtyping using 5hmC-sequencing of cell-free DNA

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Background: Epigenetic and genetic characterization of cell-free DNA (cfDNA) has the potential to non-invasively classify tumor subtypes to help guide precision oncology. In this study, we aimed to study whether 5-hydroxymethylcytosine sequencing (5hmC-seq) of cfDNA predicts gene expression and can distinguish tumor subtypes defined in matched tissue from men with metastatic castration-resistant prostate cancer (mCRPC).

Methods: We performed 5hmC-seq and low-pass whole-genome sequencing (lpWGS) of plasma-derived cfDNA from 86 mCRPC patients with matched tumor tissue that were profiled with 5hmC-seq (N=49) and RNA-sequencing (N=86). We developed a 5hmC-seq based Random Forest-based hierarchical classifier to distinguish androgen receptor (AR)- positive prostate cancer (ARPC), neuroendocrine prostate cancer (NEPC) and double-negative prostate cancer (DNPC). The previously published Griffin and ctdPheno tools were used to classify samples based on lpWGS. The locked classifiers were evaluated in a separate cfDNA cohort from men with mCRPC (N = 64).

Results: Samples with less than 10% circulating tumor DNA fraction (ct-fraction) were categorized as a good prognosis group, while the rest of the samples were used for classifier training and testing (APRC (N = 23), NEPC (N = 6), and DNPC (N = 11)). The 5hmC-based Random Forest subtype classifier reached an AUC of 0.90 [95% CI 0.78-1.0] and 0.88 [95% CI 0.77-1.0] for NEPC and DNPC prediction in cross-validation, respectively. The 5hmC-based predicted subtype in the separate validation cohort was strongly prognostic for overall survival; NEPC compared to ARPC HR = 13.9 [95% CI: 1.6 - 121.8], p = 0.017, and DNPC compared to ARPC HR = 26.5 [95% CI: 4.0 - 178.2], p =7.4 x 10-4, also after adjusting for ct-fraction. Furthermore, high 5hmC-seq NE score in cfDNA predicted shorter overall survival (HR = 13.7 [95% CI: 3.5 – 54.2], p = 2 x 10-4), whereas high 5hmC-seq AR score predicted longer overall survival (HR = 0.15 [95% CI: 0.05 – 0.41], p =2.1 x 10-4). Concurrently sequenced lpWGS demonstrated that the ratio of short (< 150 bp) vs. long (150 ~

180 bp) fragments was higher in NEPC compared to other subtypes at the similar ct-fraction (ANOVA test P = 0.0028). ARPC showed low central coverage at AR binding sites, while NEPC exhibited low central coverage at ASCL1 binding sites. DNPC displayed no signal at either binding site. The predicted subtypes based on nucleosome positioning patterns from lpWGS were concordant with that predicted by 5hmC-seq in the separate cohort.

Conclusions: The developed multi-class subtype classifiers using cfDNA showed promise in accurately predicting subtypes defined in matched tissue and were strongly prognostic. Thus, 5hmC-seq of cfDNA may non-invasively reveal tumor phenotypes, classify tumor subtypes in concordance with matched tissue, and potentially monitor tumor progression through tumor-specific signaling. Limitations include capturing sufficient fraction of tumor derived cfDNA.

Funding Acknowledgements

M. Sjöström was supported by the Swedish Research Council (Vetenskapsrådet) with grant number 2018-00382, the Swedish Society of Medicine (Svenska Läkaresällskapet), a Prostate Cancer Foundation Young Investigator Award and a Swedish Cancer Society Junior Clinical Investigator Award. R. Shrestha was funded by a Prostate Cancer Foundation Young Investigator Award. Xiaolin Zhu was funded by a Prostate Cancer Foundation Young Investigator Award. S.G. Zhao was funded by a Prostate Cancer Foundation Young Investigator Award, DOD W81XWH2110205, NIH 1DP2CA271832-01, and is a UW Carbone Cancer Center Ride Scholar. H. Li was supported by a Prostate Cancer Foundation Young Investigator Award. D.A. Quigley was funded by the UCSF Benioff Initiative for Prostate Cancer Research, the Prostate Cancer Foundation, NCI SPORE 1P50CA275741, and Department of Defense awards W81XWH-22-1-0833 and HT94252410252. E.J. Small was funded by Prostate Cancer Foundation Challenge Grant and NIHP50 CA275741-01. F.Y. Feng was funded by Prostate Cancer Foundation Challenge Awards. Additional funding was provided by a UCSF Benioff Initiative for Prostate Cancer Research award. F.Y. Feng was supported by NIH/NCI 1R01CA230516-01, 1R01CA227025, and by NIH P50CA186786. F.Y. Feng was supported by Department of Defense grant W81XWH-19-1-0682. JJA was supported by 1R01CA291986 and 1R01CA282005, a Prostate Cancer Foundation Challenge Award, and a Joint Institute for Cancer Research Award.

Conflicts of Interest Disclosure Statement

A. Foye reports personal fees from Varian Medical Systems outside this work. S.G. Zhao has patent applications with Veracyte on molecular signatures in prostate cancer unrelated to this work and has a family member employed by Artera. S. Levy, Y. Ning and G. Guler holds stock as an employee of Clearnote Health. R. Aggarwal reports grants from Janssen, Amgen, Zenith Epigenetics, and Xynomic Pharmaceuticals; grants and personal fees from AstraZeneca, Merck, and Novartis; personal fees from Dendreon, Elsevier, Exelixis, Jubilant Therapeutics, Bayer, Pfizer, and Alessa Therapeutics outside the submitted work. E.J. Small reports other support from Fortis, Harpoon, Teon, Janssen, Johnson & Johnson, and Ultragenyx during the conduct of the study; other support from Fortis, Harpoon, Teon, Janssen, Johnson & Johnson, and Ultragenyx outside the submitted work. A.W. Wyatt reports personal fees from AstraZeneca, Merck, and Janssen, and grants from ESSA Pharma outside the submitted work. F.Y. Feng reports personal fees from Janssen Oncology, Bayer, PFS Genomics, Myovant Sciences, Roivant Sciences, Astellas Pharma, Foundation Medicine, Varian, Bristol Myers Squibb (BMS), Exact Sciences, Clearnote Health, Novartis, and Tempus; other support from Serimmune and Artera outside the submitted work. M. Sjöström reports personal speaker fees from Astellas and personal consulting fees from Veracyte and Adelphi Targis outside the submitted work. J. Alumkal has received consulting fees from Fortis Therapeutics and ORIC Pharmaceuticals, and research support to his institution from Beactica and Zenith Epigenetics outside of the submitted work. His institution has also received research support from a National Comprehensive Cancer Network (NCCN)/Astellas Pharma Global Development, Inc./Pfizer, Inc. research award outside of the submitted work.