Whole genome methylation of tumor biopsies and circulating tumor DNA in advanced prostate cancer

Himisha Beltran[1], Emma Dann[2], Michael Sigouros[1], Alicia Alonso[1], David Nanus[1], Scott Tagawa[1], Jenny Xiang[1], Alessandro Romanel[2], Matteo Benelli[2,3], Francesca Demichelis[1,2]

[1] Weill Cornell Medicine. New York, NY, USA
[2] University of Trento. Trento, Italy
[3] Hospital of Prato, Istituto Toscano Tumori. Prato, Italy

Background: We previously identified mechanisms underlying the clonal evolution of castration resistant prostate adenocarcinoma (CRPC-Adeno) to a neuroendocrine resistance phenotype (CRPC-NE) and found significant epigenetic alterations in CRPC-NE characterized by changes in DNA methylation (Beltran et al, Nat Med 2016). We aim to develop a non-invasive approach to identify patients that are developing CRPC-NE.

Methods: Differential methylation analysis of large tissue –based patient cohorts (n>400) was performed for CpG islands using an AUC-based selection approach. Assessment of methylation patterns in circulating tumor DNA (ctDNA) using whole genome bisulfite sequencing (WGBS) combined with whole exome sequencing (WES) and matched germline was performed in patients with CRPC-Adeno and CRPC-NE. ctDNA (min 5ng) was bisulfite converted; single stranded DNA was processed and enriched using PCR with primers. Libraries were clustered at 12 pM on a pair end read flow cell and sequenced via Illumina HiSeq 2500 (125 cycles). WGBS raw data was quality filtered, adapter trimmed, aligned to Human genome build GRCh37/hg19. The patients ctDNA results were compared with DNA methylation and WES profiles of temporally matched metastatic tumor biopsies and the larger tissue –based patient cohorts.

Results: Differentially methylated regions across disease states allowed for subytype segregation of prostate cancer tissue samples (50 benign prostate, 340 localized prostate adenocarcinoma, 25 CRPC-Adeno, 19 CRPC-NE) and for the identification of class specific markers. CRPC-NE specific methylation patterns both hyper- and hypo-methylated identified in the study tissue cohort included markers involved in cell differentiation processes, chromatin remodeling, developmental and neuronal development pathways (TGF-BETA signaling, Notch pathway, Wnt/beta-catenin signaling). DNA methylation of ctDNA by WGBS mimicked the DNA methylation patterns of matched tumor biopsies in the same patient (Pearson coefficients: 0.78-0.91) and segregated with tumors of the same clinical disease state by principal component analysis. ctDNA methylation at CpG sites within candidate markers were consistent with trends observed in the tissue-based cohorts.

Conclusions: Combined whole genome methylation and WES of ctDNA is feasible, concordant with biopsy tumor tissue, and may help identify the spectrum and frequency of CRPC-NE genomic and epigenetic changes. These data are being extended to a larger cohort including the assessment of dynamic changes with time, progression, and treatment resistance with a goal for the early detection of CRPC-NE resistance using non-invasive epigenetic profiling.

Conflicts of Interest: None

Funding: This work was supported by the Prostate Cancer Foundation