Low Pass Whole Genome Sequencing of Single Circulating Tumor Cells for Detection of Chromosomal Instability (CIN) and Non-Coding Indels Across Multiple Solid Tumors

Angele E. Rodriguez, Jerry Lee, Ramsay Sutton, Rhett Jiles, Yipeng Wang, Mark Landers, Ryan Dittmar

Background

-Mismatch repair deficiency (MMR-D) has emerged as a robust biomarker to predict patients' response to immune checkpoint inhibitors, whereas chromosomal instability could inform responses to PARP inhibitors.
- Assessment of MMR-D and CIN in bulk tumor samples is well explored, but limited by sample accessibility and tumor heterogeneity.
- Analysis of CIN is challenging for MMR-D and CIN, especially for patients who harbor subclonal genomic alterations, limiting its clinical utility.
- Epic Sciences' CTC platform employs a non-enrichment based approach to provide insight into subclonal heterogeneity.
- Here we present a single CTC genomics assay to simultaneously detect CIN and non-coding indels by using low coverage whole genome sequencing (WGSS).

Methods

- Contrived samples were prepared by spiking prostate cancer cell lines, LNCaP, PC3 and VCaP, into healthy donor blood. Red blood cells lysed, nucleated cells deposited onto glass slides and immunofluorescently stained (DAPI, CK, CD45, and Androgen Receptor) identified cancer cells were individually isolated from the slides, lysed, whole genome amplified (WGA), shotgun library prepared, and low pass whole genome sequenced to ~ 20X coverage. Data were analyzed for non-coding indels and large scale transitions (LSTs, surrogates for MMR-D and CIN, respectively). Microsatellite instability (MSI) was measured by the Giagen Type-it MSI PCR kit as per manufacturer's protocol on the control cell lines.
- 1998 CTCs from 175 prostate, breast, bladder and lung, renal cancer patients were evaluated for clinical feasibility.

Conclusions

- We demonstrate the feasibility of detecting Indel Scores and CIN simultaneously at the single cell level using Epic Sciences CTC platform.
- Inter- and intrapatient heterogeneity was observed in this large patient cohort.
- MMR-D and HRD seem to be mutually exclusive events driving tumor genome evolution, however, we find patients where both MMR-D and HRD tumor subclones co-occur.
- Studies are ongoing to validate the potential correlations of Indels as a surrogate of MMR-D/Checkpoint inhibitor, and CIN with PARP response.

Schematic of Epic CTC platform for CTC identification, single cell genomics, and NON-Coding (INDEL) analyses workflow:

1) SLIDE PREPARATION
2) CELL STING
3) SCANNING
4) SINGLE CELL DIGITAL PATHOLOGY
5) Single Cell Isolation & CIN, INDELS & MSI

CIN and INDELS Scores of CTCs from Multiple Tumors

CIN Scores

Patients

INDEL Scores

CIN vs INDEL scores

p=0.001 (Fisher Test)

CIN and INDELS Scores of Prostate Ca Cell Lines

CIN

INDELS

CIN vs INDELS

CIN/HRD

References