The Use of Whole Genome Copy Number Variation (CNV) to Measure Genomic Instability in mCRPC CTCs

Yipeng Wang, Stephanie Greene, Angel Rodriguez, Jerry Lee, Laura Leitz, Mark Landers, Adam Jendrisak, Ryon Graf, Ryan Dittamore, Dena Marrinucci
Epic Sciences, San Diego, CA

EPIC SCIENCES
www.epicsciences.com

Background

- Epic Sciences' non-enrichment CTC analysis platform enables accurate genomic profiling of heterogeneous CTC populations with single cell resolution.
- AR targeted therapies (AR Tx) in combination with PARP inhibitors have recently shown efficacy in the treatment of mCRPC patients with specific DNA repair genes mutations detected from metastatic tissue biopsies.
- Homologous recombination DNA repair deficiencies (HRD), associated with response to PARP inhibitors, can be assessed through identification of genomic instability or scarring.
- Accurate genomic scarring measures in tumors are often confounded by intra-tumor heterogeneity and/or non-tumor DNA contamination.
- We sought to develop a genomic instability and scarring feasibility assay for mCRPC patients utilizing a non-invasive, single CTC assay.

Workflow

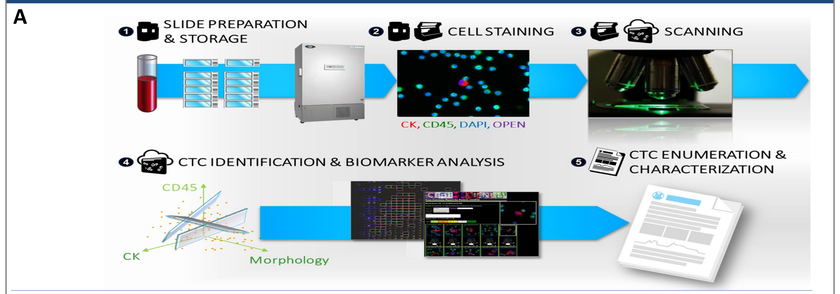


Figure 1. Methods
A. Description of standard Epic CTC analysis process (top). Images are analyzed using a multi-parametric digital pathology algorithm to detect CTC candidates and quantitate protein biomarker expression levels. CTC classifications are displayed in a web-based report and are confirmed by trained technicians.
B. Description of the CTC recovery and genomic profiling workflow (bottom). Individual cells are isolated, subjected to Whole Genome Amplification, and NGS library preparation. Sequencing is performed on an Illumina NextSeq 500.

Single Cell CNV/Genomic Instability Analysis Pipeline

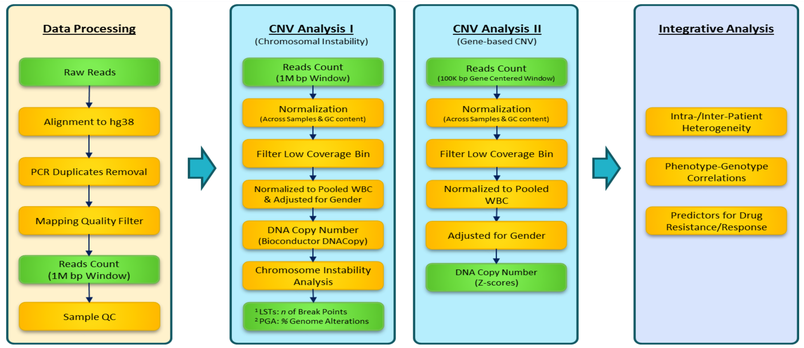


Figure 2. Bioinformatic Analysis
Raw FASTQ files are assessed and filtered for quality. Reads are aligned to the hg 38 reference genome (UCSC), PCR duplicates removed, and filtered by the MAPQ score 30. Samples with >250K reads post filtering are analyzed for copy number alterations. The filtered alignment files are further analyzed with Epic's Copy Number Pipelines. One pipeline was for estimating genomic instability using 1M bp window, and the other was for gene specific copy number measurement.
[1] LSTs: n of chromosomal breaks between adjacent regions of at least 10 Mb. [2] PGAs: percentage of a patient's genome harboring copy number alterations (amplification or deletions).

Whole Genome CNV in Single Cells

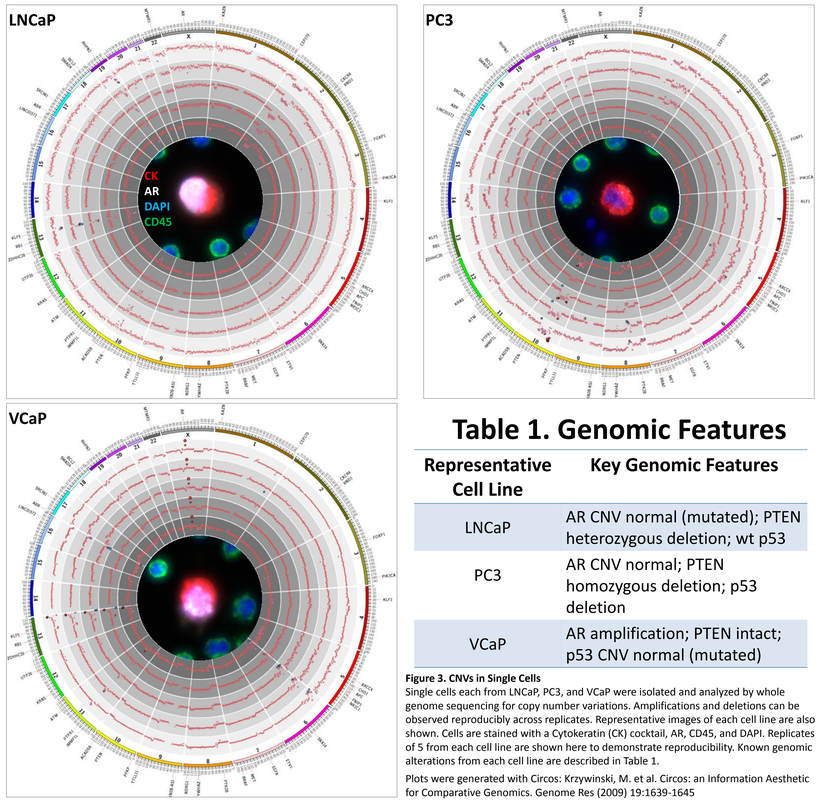


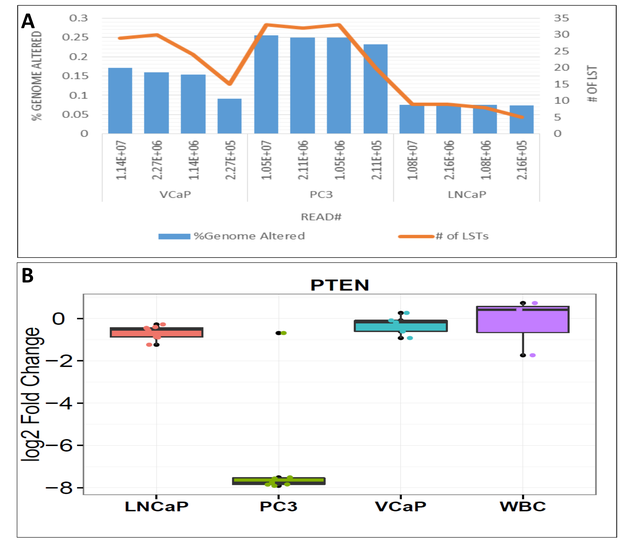
Table 1. Genomic Features

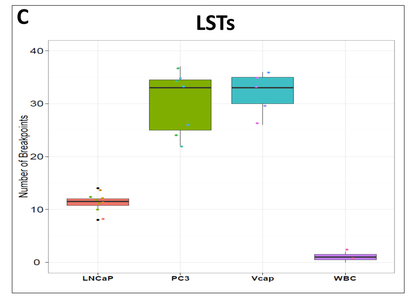
Representative Cell Line	Key Genomic Features
LNCaP	AR CNV normal (mutated); PTEN heterozygous deletion; wt p53
PC3	AR CNV normal; PTEN homozygous deletion; p53 deletion
VCaP	AR amplification; PTEN intact; p53 CNV normal (mutated)

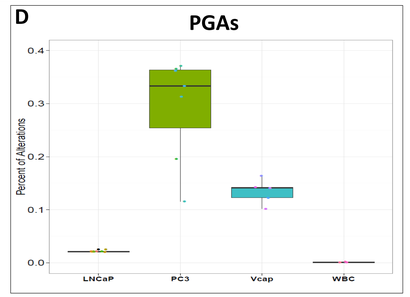
Figure 3. CNVs in Single Cells
Single cells each from LNCaP, PC3, and VCaP were isolated and analyzed by whole genome sequencing for copy number variations. Amplifications and deletions can be observed reproducibly across replicates. Representative images of each cell line are also shown. Cells are stained with a Cytokeratin (CK) cocktail, AR, CD45, and DAPI. Replicates of 5 from each cell line are shown here to demonstrate reproducibility. Known genomic alterations from each cell line are described in Table 1.
Plots were generated with Circos: Krzywinski, M. et al. Circos: an Information Aesthetic for Comparative Genomics. Genome Res (2009) 19:1639-1645

Genomic Instability in PCa Cell Lines

Figure 4. Copy Number Variation and Genomic Instability Measurements
A. Comparison of read number vs. detection of genomic instability. Following Q30 filtering, representative cell FASTQ files were randomly subsampled reducing the number of reads from ~20M to 0.25M to determine the minimum number of reads required to detect LSTs and preserve the % genome altered.
B. Comparison of log2 copy number for PTEN for each replicate (n=5) cell across 3 PCa cell lines and WBC control.
C. Comparison of the # of breakpoints (LSTs) for each replicate (n=5) cell across 3 PCa cell lines and healthy donor WBC control. Each value is plotted individually, boxplots represent upper and lower quartile and the line represents the median value.
D. Comparison of the % genome altered (PGA) for each replicate (n=5) cell across 3 PCa cell lines and healthy donor WBC control. Each value is plotted individually, boxplots represent upper and lower quartile and the line represents the median value.







Conclusions

- Whole genome copy number analysis can be used to reproducibly characterize genomic instability by measuring LSTs and PGA
  - ≥1M reads/cell low pass whole genome sequencing required to detect genomic instability signature across replicate PCa cells
  - %cv # of CNV ranged detected ranged from 7-20% across replicate cells
  - %cv # of LSTs detected ranged from 12-20% across replicate cells
  - Median of 4 LSTs/cell with less than <1% PGA detected in WBC control
- Highest genomic instability associated with p53 mutation and PTEN deletion
  - Highest # of LSTs detected in PC3 & VCaP, both harbor p53 alterations
  - Highest % genome altered detected in PC3
    - PTEN null
    - p53 deletion