Phenotypic Profiling of Circulating Tumor Cells (CTCs) in Patients with Metastatic Breast Cancer Reveals Clinically-Relevant Heterogeneity in CTC Morphology and Marker Expression

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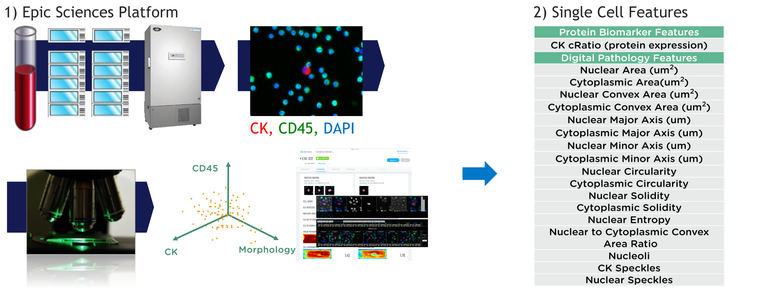
BACKGROUND

- The choice between hormonal therapies and chemotherapy is a frequent decision in the care of metastatic breast cancer (mBCa) patients.
- We previously developed quantitative measures of phenotypic CTC heterogeneity in metastatic castration resistant prostate cancer (mCRPC), and found higher heterogeneity was associated with better survival on chemotherapy vs. targeted hormonal therapies, and the reverse was true in low heterogeneity patients (Scher et al. 2017 Cancer Research).
- We sought to apply our previous heterogeneity quantitation methodologies to a cohort of mBCa patient CTCs to ascertain feasibility in mBCa.

METHODS

- 295 blood samples from mBCa patients were processed for CTC analysis utilizing the Epic Sciences platform. Following enumeration, multi-dimensional phenotypic characterization analysis was performed utilizing protein expression and digital pathology features.
- Features from each CTC (3760 CTCs from 165 patients, 84 HR+, 19 Her2+, 8 HR+/Her2+, 54 TNBC) were compared by unsupervised clustering, Shannon Index and intra-patient variance analyses to assess the intra-patient heterogeneity among mBCa CTC phenotypes.



Schematic of Epic CTC Platform CTC enumeration, morphology, biomarker analyses and single cell sequencing workflow:

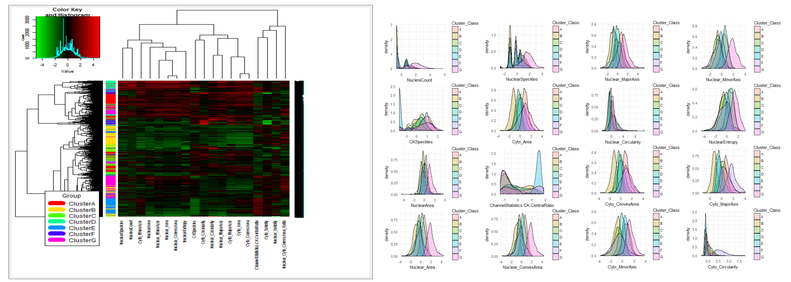
1. Nucleated cells from blood sample placed onto slides and stored in a -80◦C biorepository. Slides are stained with cytokeratin (CK), CD45, DAPI and scanned. CTC candidates are detected by a multi-parametric digital pathology algorithm followed by human reader confirmation of CTCs and quantification of biomarker expression.
2. CTCs are segmented within the DAPI and CK channels and single cell features are extracted.
3. CTCs undergo Principle Component Analysis (PCA) removing noise and redundant dimensions, and weighing features with more variance. Machine learning clustering algorithms found 7 CTC subtypes from macro trends in high-dimensional biomarkers across all CTCs from all samples in cohort, and assigned each CTC to 1 of 7 subtypes (letters A through G). Heterogeneity is quantified by counting CTCs per "Cell Type" in each sample, then using a standard Shannon Index to quantify CTC phenotypic diversity per patient sample.
4. Single cells are identified, relocated, captured, whole genome amplified (WGA), library prepared and low pass whole genome sequenced for Large Scale Transitions (LST, a surrogate of chromosomal instability) and gene copy number alterations (CNA) (Greene et al. 2016 Plos One).

References:

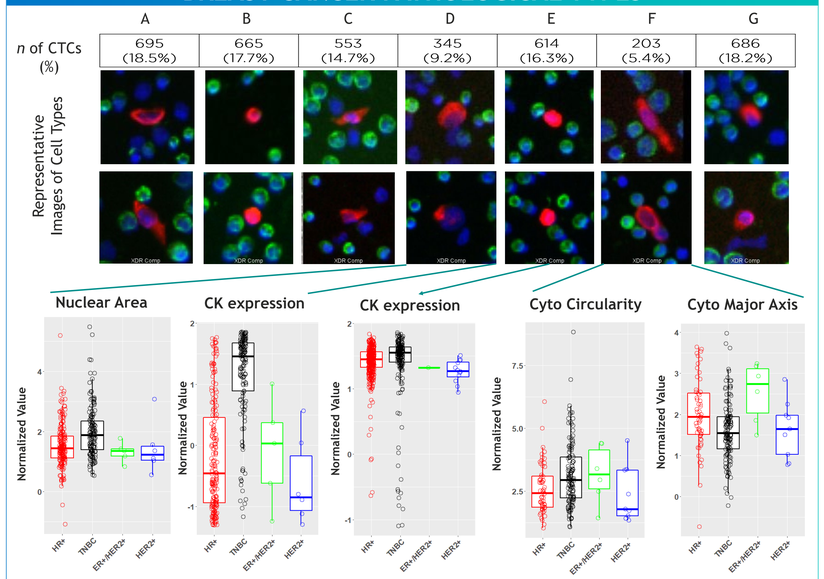
Scher HI, et al. Phenotypic Heterogeneity of Circulating Tumor Cells Informs Clinical Decisions between AR Signaling Inhibitors and Taxanes in Metastatic Prostate Cancer. Cancer Res. 2017 Oct 15;77(20):5687-5698

Greene SB, et al. Chromosomal Instability Estimation Based on Next Generation Sequencing and Single Cell Genome Wide Copy Number Variation Analysis. PLoS One. 2016 Nov 16;11(11):e0165089.

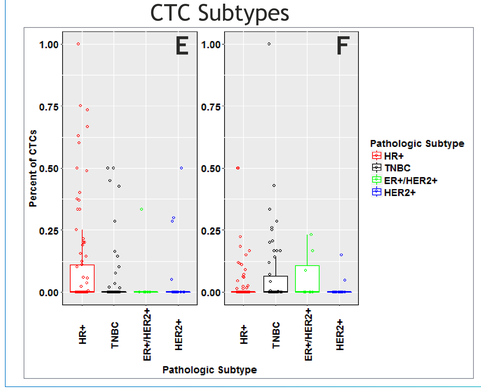
CTC SUBTYPES CLUSTER ALONG MORPHOLOGICAL FEATURES



MORPHOLOGICAL FEATURES OF CTC SUBTYPES CHARACTERIZE BREAST CANCER PATHOLOGICAL TYPES

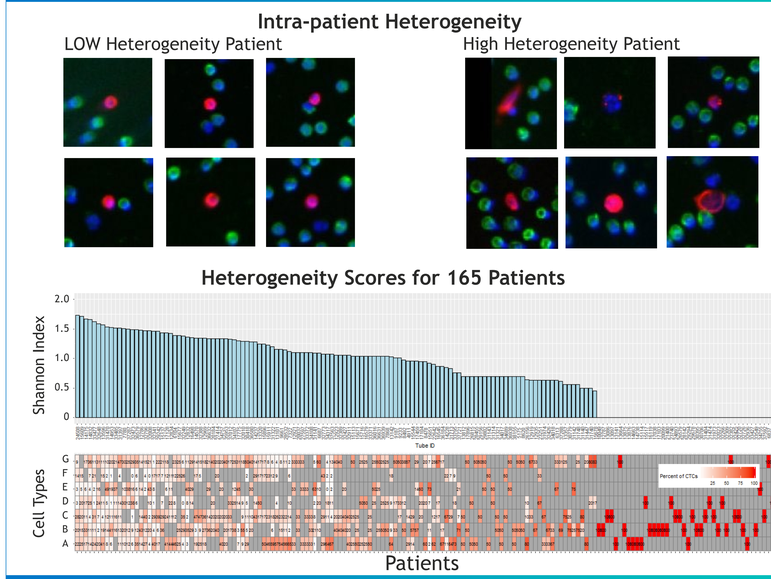


CTC SUBTYPES BURDEN DIFFERENTIATE BREAST CANCER PATHOLOGICAL TYPES

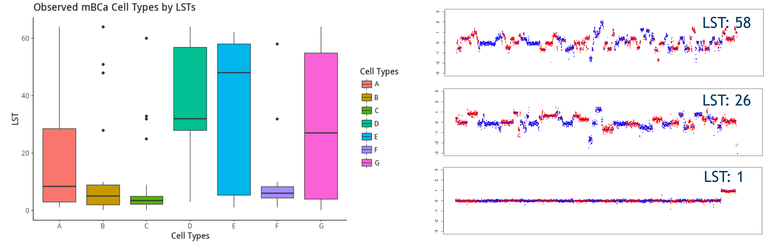


- HR+ BCa displays:
  - Enrichment in E subtype
  - Highest CK expression in E
  - Low nuclear area on average
  - Low cyto circularity (not in F)
- TNBC BCa displays:
  - Enrichment in F subtype
  - Highest CK expression in D, E
  - High circularity in F
  - Highest nuclear area in D
- ER+/HER2+ BCa displays:
  - Enrichment in F subtype
  - Low CK expression on average
  - High cyto circularity
  - High cyto major axis

PHENOTYPIC HETEROGENEITY AND QUANTITATION



UNIQUE GENOMIC PROFILES OBSERVED FOR CTC SUBTYPES



	A	B	C	D	E	F	G
n **of CTCs Sequenced**	14	21	22	30	27	11	13
ERBB2 Gain	14%	5%	0%	30%	33%	9%	46%
FGFR1 Gain	29%	52%	18%	37%	70%	18%	38%
BRCA1 Loss	7%	0%	5%	20%	15%	0%	15%
BRCA2 Loss	14%	14%	14%	33%	44%	18%	31%
CDH1 Loss	43%	10%	27%	83%	63%	27%	77%
PTEN Loss	21%	14%	18%	3%	0%	18%	0%
TP53 Loss	29%	14%	23%	83%	67%	27%	54%

CONCLUSIONS

- Distinct CTC phenotypes can be visualized reproducibly across patients and associate with specific mBCa subtypes.
- We detected a wide range of inter- and intra- patients heterogeneity in CTC phenotypes.
- Stratification of patients based on heterogeneity levels may help to find better responders to different targeted therapies.
- CTC phenotypes are associated with chromosomal instability and loss/gain of cancer driver genes.
- High CK expression in CTC subtypes D, E and G seem to correlate with higher chromosomal instability.
- Studies linking heterogeneity to therapeutic efficacy and patient outcome are ongoing.