USC Norris Comprehensive Cancer Center

Part of the Keck School of Medicine of USC

The whole transcriptional landscape of circulating tumor cells compared to metastases in stage IV breast cancer

Alexander Ring, Tania B. Porras, Daniel Campo, Pushpinder K. Bains, Victoria Forte, Debu Tripathy, Janice Lu, Gabriel Zada, Naveed Wagle, Julie E. Lang

Background

Metastasis is responsible for the vast majority of breast cancer related deaths. Metastatic breast cancer (MBC) is inherently different than primary breast cancer (BC), evolving under selection pressure at different organ sites or during systemic therapy. The current ASCO guidelines call for biopsy of a metastatic site to guide decision making for systemic therapy. Yet, biopsies of macro metastasis are oftentimes not feasible in the clinical setting. Circulating tumor cells (CTCs) have been shown to be prognostic in MBC, but their use as clinical biomarker beyond CTC enumeration has been limited. A better understanding of CTC-biology compared to metastasis may shed light on treatment opportunities and help advance the application of CTCs as liquid biopsies in clinical practice. The ANGLE Parsortix system is a microfluidics device that separates CTCs based on size and deformability, without the need for cell-surface marker selection. Our lab has previously demonstrated the feasibility of gene expression profiling of rare CTCs. **Here, we evaluated whether whole transcriptome sequencing (RNA Seq) gene expression profiling of ANGLE Parsortix isolated CTCs may serve as a surrogate for biopsies of macro metastases.**

Methods

CTCs from 21 MBC patients were enumerated and captured from 10mL peripheral blood (PB) via the ANGLE Parsortix system. RNA Seq was performed on fresh metastatic tumor biopsies (mets), CTCs and peripheral blood (PB) from all patients. Biopsy sites included: skin (n=1), lung (n=1), pleural effusion (n=5), pericardial effusion (n=1), breast (n=3), lymph node (n=2), brain (n=4), liver (n=1), ascites (n=3), cerebrospinal fluid (n=2), bone tissue (n=1). 19/21 patients were included in subsequent data analysis. **(A) Group comparison** of biologically relevant gene expression patterns in CTCs, mets and PB was performed. **(B) Differential expression of genes of interest (oncogenes, breast cancer related genes, mesenchymal and cancer stem cell (CSC) genes)** between CTCs, mets and PB was investigated. **(C) Survival analysis** based on gene expression in CTCs and mets compared to PB was performed using data from The Cancer Genome Atlas (TCGA). **(D) Single nucleotide variants (SNV) analysis** using IGV was performed in corresponding CTCs/mets pairs. **(E) Clinically actionable gene (n=66) expression and molecular signaling pathways (n=7)** for each patient were explored. **(F) Intra-patient serial time points** were analyzed, and detailed clinical-pathological and treatment data was evaluated.

Conclusion

We present the whole transcriptomic landscape of CTCs with comparison to metastases and peripheral blood all acquired prior to treatment of Stage IV breast cancer. Multiple genes, including oncogenes, breast epithelial, mesenchymal genes and CSC genes, were found with higher expression in CTCs versus metastases. When focusing on 66 known potentially clinically actionable genes in breast cancer, represented by 7 molecular signaling pathways, CTCs did not show significantly different patterns of expression versus mets compared to PB. Longitudinal analysis of 4 patients over time who had serial CTC assessments showed changing biological characteristics of CTCs isolated at different time points during treatment and disease progression. RNA Seq of CTCs may be utilized to identify molecular alterations in MBC patients that are potentially clinically actionable.

Results

(A) Whole transcriptome RNA Seq gene expression - group analysis

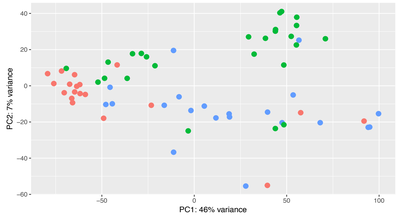


Figure 1: Principal component (PC) analysis of CTCs, Mets and PB. The results show separation of the majority of CTCs versus mets and PB in PC1, and separation of CTCs and mets from PB in PC2.

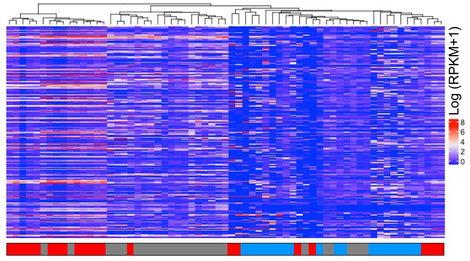


Figure 2: Differential gene expression analysis of CTCs, mets and PB. Two dimensional hierarchical clustering of all samples based on a 253 gene signaturRe that distinguishes CTCs (blue), mets (grey) and PB (red) (FDR adjusted p<0.05).

(B) Differential expression of genes of interest

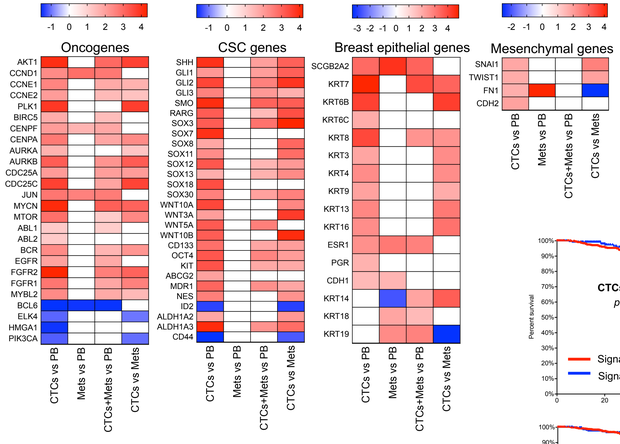


Figure 3: Expression of genes of interest in CTCs or mets compared to PB: CTCs as a group showed much stronger gene expression of oncogenes, stem cell genes, keratins and mesenchymal markers than did mets from the same patients.

(C) Survival analysis

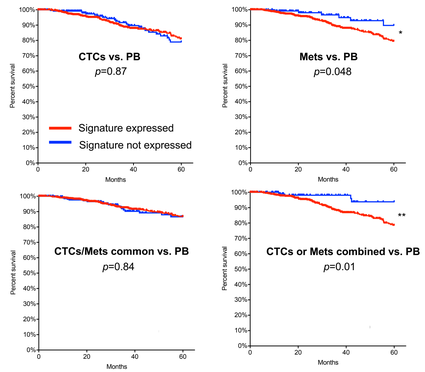


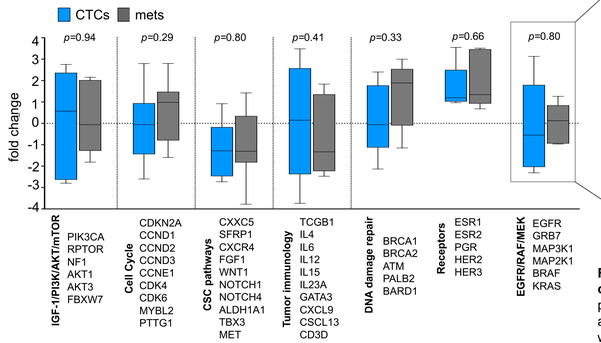
Figure 4: TCGA BC (n=817) overall survival (OS) based on 50-gene expression signatures. The top 50 highest expressed genes in four comparison conditions are shown. The 50-gene signature expressed CTCs and/or mets was superior to all other comparisons in predicting poor OS (p=0.01).

(D) Single nucleotide variants (SNV) analysis

Patient ID	SNVs in ESR1	Rs number in dbSNP
78536_CTC	T>C chr6:152,097,179	not found
78536_MET		
79412_CTC	T>C chr6:152,099,995	dbSNP: rs3798577
79412_MET		
101738_CTC_FOLLOWUP	G>T chr6:152,101,052 C>T chr6:152,101,200	dbSNP: rs72993667 dbSNP: rs2747648
101738_MET		
36541_CTC	C>A chr6:152,098,960 C>T chr6:152,101,200	dbSNP: rs2228480 dbSNP: rs2747648
36541_MET_BREAST		

Table 1: SNVs common to all CTC/met pairs in ESR1 and ERBB2: Using IGV genome viewer, six SNVs in ESR1 (n=4 patients) were identified that are shared between CTCs and distant metastases. Five are listed in dbSNP. From those listed, **rs3798577** has been associated with increased and decreased breast cancer risk in White and Asian women, respectively. **rs2228480** is linked to decreased BC risk in white women. **rs2747648** occurs in the miR-453 binding site resulting in higher ESR1 protein expression. Known and novel SNVs were identified with RNA Seq.

(E) Clinically actionable genes/ signaling pathways



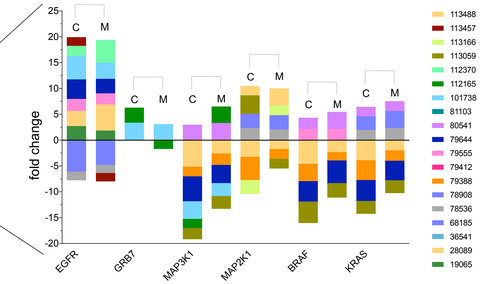


Figure 5: Analysis of potentially clinically actionable genes in breast cancer. 5A: Comparison of overall gene expression in different druggable pathways between CTCs and mets. We queried for n=66 potentially clinically actionable genes and on paired T tests for n=9 (7 shown here) pathways there was no significant difference in mean gene expression between CTCs and metastases. **5B:** Expression of clinically actionable target genes in CTCs and mets per patient for the EGFR/RAF/MEK pathway (C – CTCs, M – mets).

(F) Intra-patient analysis

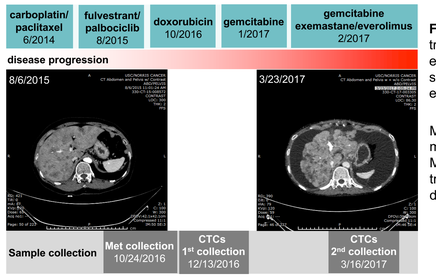


Figure 6: Intra-patient (n=1) two time-point comparison: 6A: Clinical data (including treatment and imaging studies) and sample collection are shown. **6B:** differentially expressed breast cancer genes (KEGG pathway) in met (ascites), 1st CTC and 2nd CTC sample were analyzed using IPA pathway analysis tool, demonstrating differential gene expression and pathway activation.

Metastatic site profiled: acsites, ER/PR+,HER2-. Other sites of metastatic disease: liver, lung, pleural effusion and bone. Molecular profiling with RNA Seq was done to evaluate for potential treatment targets in CTCs as a liquid biopsy as well as metastatic disease.