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 D. Nadeau, Naved 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), evoking distinct mechanism and treatment. Despite advances in therapeutic regimens, the current ASCO guidelines call for biopsy of a metastatic site to guide decision-making for systemic therapy. While biopsy of metastasis are essential for histological diagnosis, circulating tumor cells (CTCs) have been proven to be prognostic for MBC, but their role in clinical decision beyond CTC enumeration has been limited. A better understanding of CTC biology may identify novel opportunities and help advance the application of CTCs as liquid biopsies in clinical practice. The ANGEL protocol is a microfluidic device that separates CTCs that could be made based on size and deformability, without the need for cell-based marker selection. Our work has previously demonstrated the feasibility of gene expression profiling of MBC metastases.

Methods

CTCs from 21 MBC patients were enumerated and captured from 10ml peripheral blood (PB) via the ANGEL system. mRNA was extracted from PB and CTC isolated cells. RNAseq was performed on MBC tissue normalized to breast cancer excluded (PB) and for CTCs and metastases. Patients were equally divided into A, B, C (n=10) based on biological relevance of gene expression patterns in CTCs, metastases, and PB.

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

Results

We present the whole transcriptional landscape of CTCs with comparison to metastases and peripheral blood isolated prior to treatment of Stage IV breast cancer. Multiple genes, including oncogenes, breast cancer related genes, and mesenchymal (CSC) genes, were found with higher expression in CTCs versus metastases. When focusing on 60 given genes, CTCs vs. PB were compared to PB was performed using data from The Cancer Genome Atlas (TCGA) to create matching PRS.

(B) Differential expression of genes of interest

Comparisons between metastases, CTCs, and PB were investigated. (C) Survival analysis based on gene expression in CTCs and metastases compared to PB was performed using data from The Cancer Genome Atlas (TCGA) to create matching PRS.

(E) Clinically actionable genes/ signaling pathways

We identified the most relevant genes from our data set and compared them to existing druggable genes from the CTCAE (Cancer Therapy Evaluation Program) database.

Discussion

We report on the whole transcriptional landscape of CTCs with comparison to metastases and peripheral blood isolated prior to treatment of Stage IV breast cancer. Multiple genes, including oncogenes, breast cancer related genes, and mesenchymal (CSC) genes, were found with higher expression in CTCs versus metastases. When focusing on 60 given genes, CTCs vs. PB were compared to PB was performed using data from The Cancer Genome Atlas (TCGA) to create matching PRS.

Conclusion

We present the whole transcriptional landscape of CTCs with comparison to metastases and peripheral blood isolated prior to treatment of Stage IV breast cancer. Multiple genes, including oncogenes, breast cancer related genes, and mesenchymal (CSC) genes, were found with higher expression in CTCs versus metastases. When focusing on 60 given genes, CTCs vs. PB were compared to PB was performed using data from The Cancer Genome Atlas (TCGA) to create matching PRS.

Table: SNVs common to atCtCin pairs in ESRI and ESRRB.

<table>
<thead>
<tr>
<th>Patient</th>
<th>SNV in ESRI</th>
<th>SNV in ESRRB</th>
<th>ESRI vs. ESRRB</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>T&gt;C (chr15:123,456,789)</td>
<td>G&gt;A (chr15:123,456,789)</td>
<td>not found</td>
</tr>
<tr>
<td>B1</td>
<td>C&gt;T (chr15:123,456,789)</td>
<td>A&gt;G (chr15:123,456,789)</td>
<td>not found</td>
</tr>
<tr>
<td>C1</td>
<td>G&gt;A (chr15:123,456,789)</td>
<td>T&gt;C (chr15:123,456,789)</td>
<td>not found</td>
</tr>
</tbody>
</table>

Figure 1: Principal component (PC) analysis of CTCs, PB, and MBC. The results are on the mean values across samples for CTCs and MBCs.

Figure 2: Differential gene expression analysis of CTCs, MBC, and PB. Two-dimensional hierarchical clustering of all samples based on a 2304 gene signature identified in CTCs (blue), metastases (red), and PB (not).

Figure 3: Expression of genes of interest in PB, CTCs, and MBCs. A group showed much stronger gene expression of oncogenes, stem cell genes, keratin, and mesenchymal markers than did PB from the same patients.

Figure 4: TCGA-BC (n=111) overall survival (OS) based on 50 gene expression signatures. The top 50 highest expressed genes in PB were compared between CTCs and metastases. For five given genes in the TCGA-BC dataset, OS was associated with increased and decreased breast cancer risk.

Figure 5: Analysis of potentially clinically actionable genes in breast cancer. 5A: Comparison of overall gene expression in different droppable pathways between CTCs and PB. We ranked all potentially actionable genes and calculated the list of potentially actionable genes and then tested the list for enrichment. Pathways there were no significant difference in mean gene expression. 5B: Pathway analysis tool, demonstrating differential gene expression and pathway activity.

Figure 6: Intra-patient (PB) time-point comparison: 6A: Clinical data (including treatment and imaging studies) and sample collection are shown. 6B: differentially expressed breast cancer genes (DEG) pathway with PB and MBC. PB and MBC were compared using DEG pathway analysis tool, demonstrating differential gene expression and pathway activity.