

Increased chromatin heterogeneity in circulating tumor cells (CTCs) is associated with high levels of HER2 expression in metastatic breast cancer (MBC) (2019 AACR Abstract #1919)

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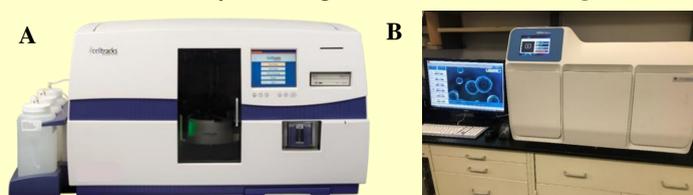


Introduction

- Circulating tumor cells (CTCs) play a critical role in the process of tumor metastasis, even after systemic treatments including surgery, chemotherapy and radiation. We previously reported that overexpression of HER2 in CTCs is associated with detection of clusters, more aggressive clinical features and prognosis in MBC (2018 AACR #5195).
- Meanwhile, alterations in chromatin nano-architecture is one of the earliest events in neoplastic transformation, which occurs during field carcinogenesis is noted in a variety of cancer types. Dr. Backman have demonstrated that Partial Wave Spectroscopic (PWS) related high order chromatin abnormalities can be used to predict risk of carcinogenesis in lung, colon, esophagus, ovarian and prostate cancer.
- Herein, we report a new finding that a widespread variation on chromatin distribution in CTCs is associated with HER2 expression in MBC which may indicate more aggressive tumor, and help to understand MBC metastasis and predict treatment benefit, especially for metastatic or recurrent disease, and it is potential utilization in clinic.

Instruments:

- CellSearch® System (Figure 1A): We Semi-automated fluorescence CELLTRACKS ANALYZERII with antibodies targeting EpCAM for capturing CTCs for CTCs enrichment.
- We use DEPArray for single –cell isolation (Figure 1B)



- We used Parsortix system for live CTCs enrichment and isolation (Figure 1C)

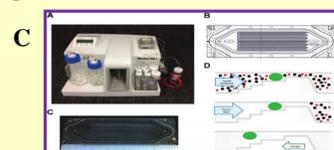


Figure 1 Major instruments we used for this study

Materials and Methods

- **Patients:** Whole blood sample (7.5ml/each) was collected from stage III/IV BCa patients before therapy at the Northwestern Memorial Hospital in 2017 that had longitudinally detection of circulating tumor cells (CTCs).
- **CTC enrichment and enumeration:** We used FDA approved CELLTRACKS ANALYZERII® System (Figure 1) and CELLSEARCH® CXC Kit contains antibodies targeting the Epithelial Cell Adhesion Molecule (EpCAM) antigen for capturing CTCs, Anti-CK-PE which is specific for the intracellular protein cytokeratin in epithelial cells, DAPI stains the cell nucleus, anti-CD45-APC is specific for leukocytes, and anti-HER2/neu-FITC is specific for HER-2/neu antigen.
- **Parsortix™ System:** Live CTCs enrichment was performed by using Parsortix system (ANGLE) utilizes microfluidic based 10µm separation cassettes(Figure.2).The captured CTCs were stained by Anti-CK-PE for epithelial cells, DAPI for nucleus, anti-CD45-APC for leukocytes, and anti-HER2-FITC
- **Partial Wave Spectroscopic (PWS) microscopy (Figure 2):** CTCs chromatin packing density was scanned by Partial Wave Spectroscopic (PWS) microscopy which is a label-free spectroscopic microscopy method that resolves structures in cells and quantify the cell nucleus heterogeneity of chromatin packing density scaling between 20-350 nm, or from the kb-pair to 10 Mb-pair range. For each nuclei, the nanoscale heterogeneity of chromatin packing was analyzed as nuclear statistical parameter standard deviation Σ (sigma, RMS)

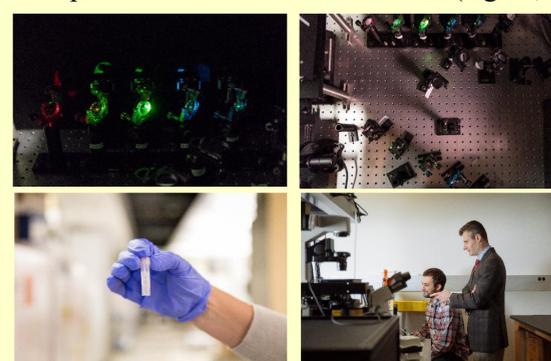


Figure 2. Partial Wave Spectroscopic (PWS) microscopy equipment in Backman's lab

- **Statistics:** Kruskal-Wallis test was used for statistics.

Results

- We identified 400 CTCs by CellSearch system, including 115 HER2⁺ CTCs and 285 HER2⁻ CTCs (Figure 4).
- The live CTCs were sorted by Parsortix system and were processed with multiple staining and scanning by PWS microscopy. CTCs were classified based on morphology and correct phenotype as CK+, DAPI+ and CD45⁻. There were 97 acquired nuclei that were scanned and evaluated successfully, including 17 HER2⁺ CTCs, 12 HER2⁻ CTCs and 80 non-CTC cells (Figure 3).
- According to the images analysis, stronger nanoscopic variations at each pixel in internal structure represents higher chromatin distribution heterogeneity quantified by RMS. **The average RMS was 0.05125 in CTC cells which is significantly higher than the non-CTC population (RMS=0.0381, p<0.01) (Figure 4).**
- Moreover, the **HER2⁺ CTCs demonstrated the highest nuclear sigma (RMS=0.05366) among all the subgroups when compared to HER2⁻ CTCs (RMS=0.04546, p<0.01) (Figure 4).**

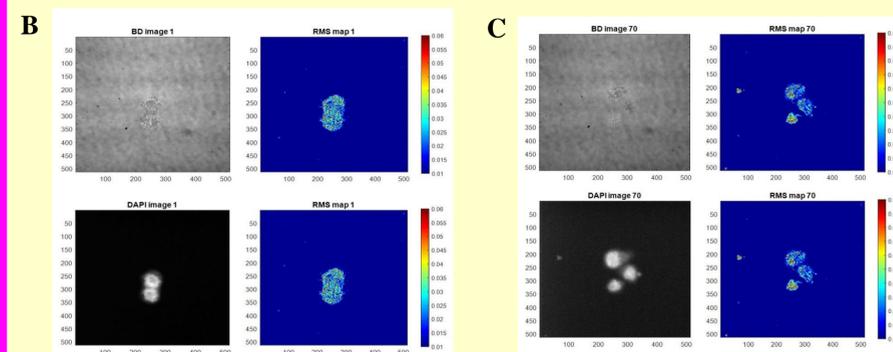
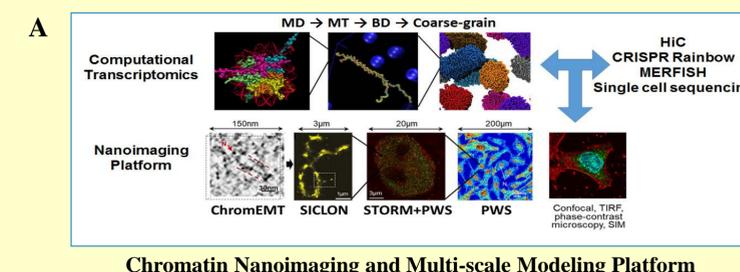


Figure 3. A. Chromatin Nanoimaging and Multi-scale Modeling Platform (from Dr. Backman's group); B The signal is scanned base on 500-700 nm wavelength (about 100 wavelengths). BD images are based on 550 nm wavelength, with Pixel 512x512, More red means more difference on distribution of stuffs (protein or chromatin et al). More difference may indicate more aggressive tumor, or more changes or specific expression under the therapeutics

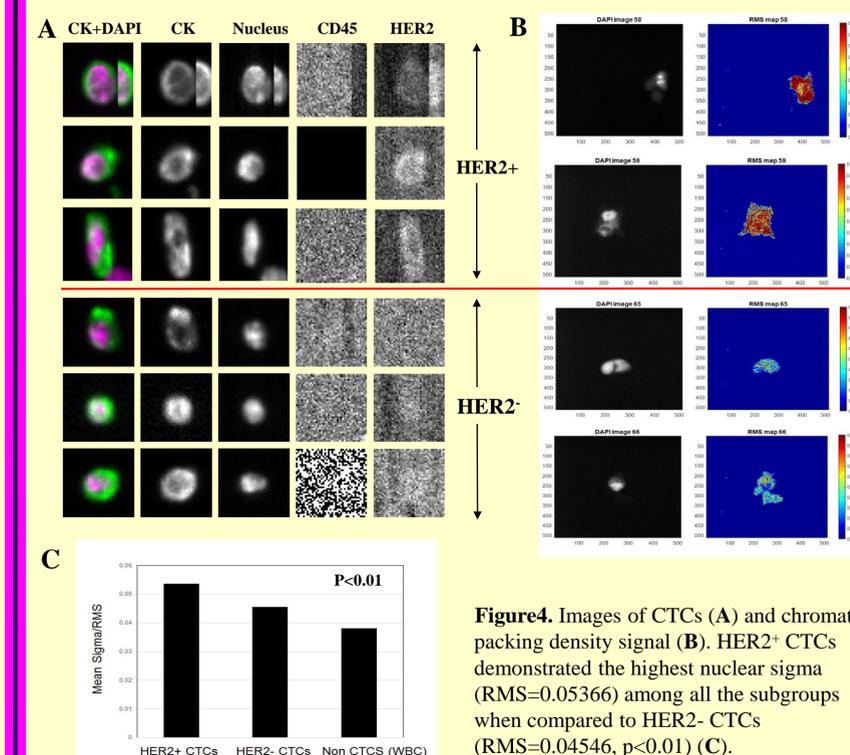


Figure 4. Images of CTCs (A) and chromatin packing density signal (B). HER2⁺ CTCs demonstrated the highest nuclear sigma (RMS=0.05366) among all the subgroups when compared to HER2⁻ CTCs (RMS=0.04546, p<0.01) (C).

Conclusions and Implications

- This is the first report evaluating chromatin heterogeneity in CTCs demonstrating the ability of using PWS microscopy for chromatin analysis
- High RMS indication of higher chromatin distribution heterogeneity is associated with more aggressive phenotypes such as CTCs HER2 expression ,
- This novel property of CTCs requires further evaluation and validation but, it may offer a novel dimension in the understanding of the metastatic process.

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