Extracellular vesicles from young women's breast cancer patients drive increased invasion of non-malignant cells via the Focal Adhesion Kinase pathway: a proteomic approach

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Background

Young women's breast cancer (YWBC) affects nearly 27,000 American women under 45 per year, and these patients are more likely to present with poor prognostic disease and have worse clinical outcomes. Extracellular vesicles (EVs) are small, cell-derived membranous structures that contribute to cancer progression and metastases by transporting biologically significant proteins, metabolites and nucleic acids. They may also serve as biomarkers of various disease states or important therapeutic targets. Breast cancer derived EVs have the potential to change the behavior of other cells in their microenvironment. However, the proteomic content of EVs isolated from YWBC patients and the mechanisms underlying the influence of EVs on tumor cell behavior have not yet been reported. The Focal Adhesion Kinase (FAK) pathway plays an important role in tumor cell migration and invasion. Previous studies have reported elevated levels of FAK in cancer-derived EVs, however, a functional link between FAK signaling and the phenotypic effects of breast cancer EVs has not previously been demonstrated.

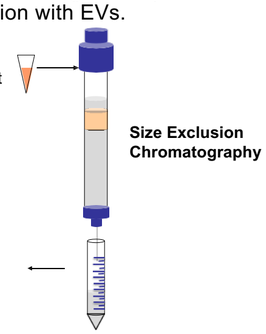
Hypothesis

EVs from YWBC patients have unique protein content which increases their contribution to the invasive properties of breast cancer cells.

Methods

We compared the proteomic content of EVs isolated from invasive breast cancer cell lines and plasma samples from young women's breast cancer (YWBC) patients with age-matched healthy donors using mass spectrometry. We analyzed the functionality of EVs in two dimensional tumor cell invasion assays and the gene expression changes in tumor cells after incubation with EVs.

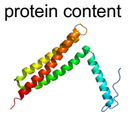
- Conditioned media of breast cancer cell lines
  - MDA231
- Human Serum
  - Healthy donors
  - YWBC patients



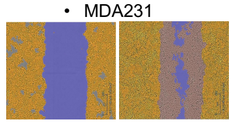
Size Exclusion Chromatography

EV concentration and **size** determined via nanoparticle tracking analysis (NTA) using the NanoSight instrument

Proteomics
- 20 YWBC patients
- 10 Healthy Donors
- 10 YWBC patients
- MetaboAnalyst used to identify significant differences in protein content



Invasion Assays
- Scratch-Wound migration of DCIS Cells treated with EVs from
  - Healthy Donors
  - YWBC patients
  - MDA231



Multiplex Gene Expression
- Gene expression panels of DCIS Cells treated with EVs from
  - Healthy Donors
  - YWBC patients
  - MDA231

nanoString

Results

YWBC EVs Promote Increased Tumor Cell Invasion

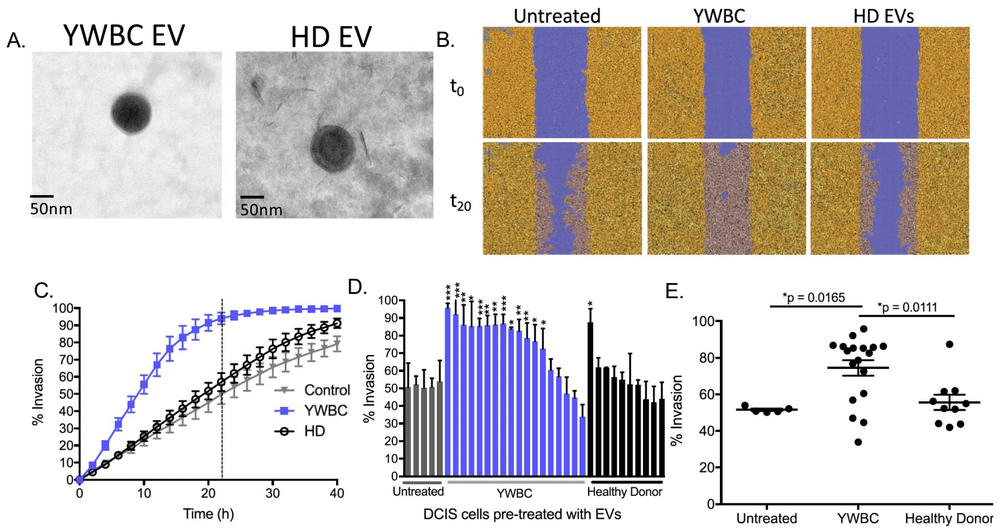


Figure 1:EVs isolated from YWBC patients promote increased invasion of DCIS cells in a scratch wound assay through a Matrigel pad. **A**. Electron microscope images of YWBC EV and HD EV. **B**. Representative images of invasion assay **C**. Average percent invasion over time **D**. Individual sample treatment average percent invasion at the time point when untreated controls reached 50% confluence **E.** The average percent invasion is significantly higher in EVs treated with YWBC EVs than HD EVs or untreated cells

YWBC EVs Have Unique Proteomic Content

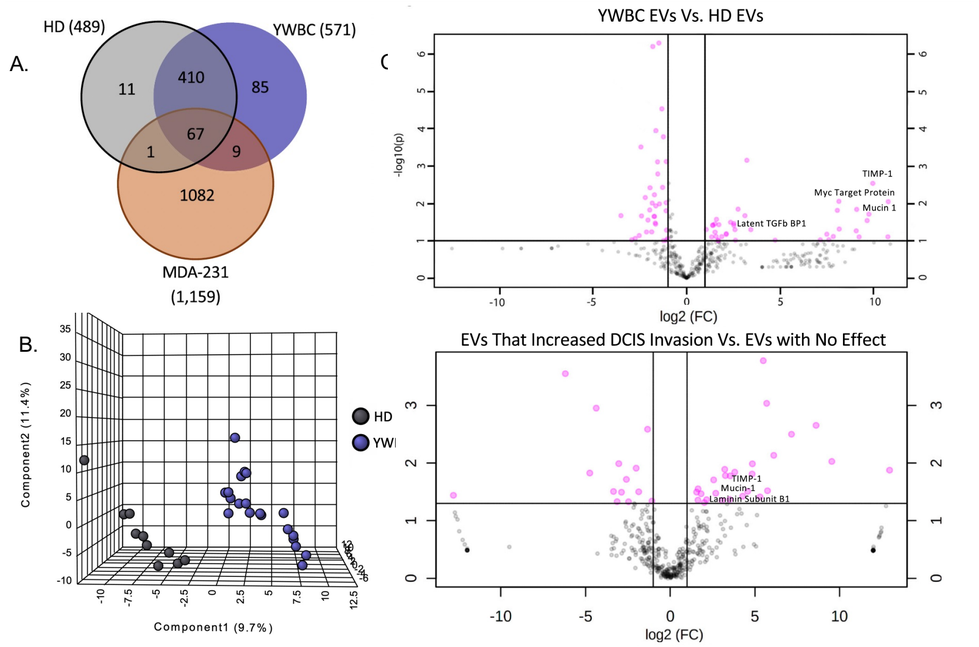


Figure 2: EVs from 20 YWBC patients and 10 healthy doanors were analyzed by mass spectrometry and identified proteins were analyzed using MetaboAnalyst 3.0 software. **A.** Vin diagram of the protein content of EVs isolated from HD, YWBC patients and MDA-231 cells. **B.** Multivariate analysis using the partial least squares discriminant analysis method distinguishes YWBC (blue) from HD (black) **C.** Volcano plot analysis demonstrates proteomic differences between EVs from YWBC patients and HD **D**. EVs with functional activity (Fig. 2) have distinct proteomic content from those without functional activity

YWBC EVs Alter Gene Expression Related to Cell Invasion

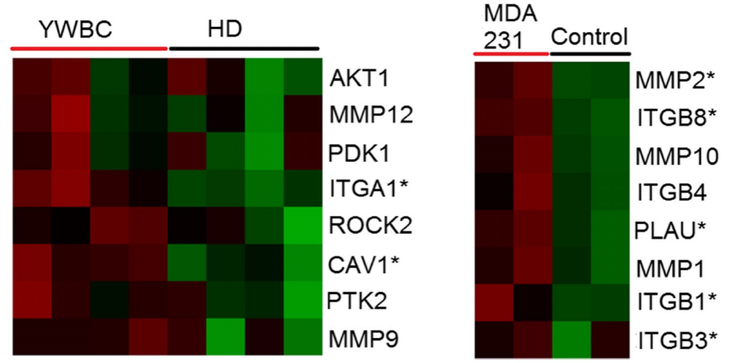


Figure 3: DCIS cells treated with BC EVs have altered gene expression related to FAK pathway. Gene expression related to cancer pathways and cancer progression was measured using NanoString technology. Heatmap shows the top 8 genes related to the FAK pathway comparing MCF10DCIS.com cells treated with EVs from YWBC patients to HD (left) or from invasive MDA231 breast cancer cells to untreated controls (right), *p < 0.05

FAK pathway inhibition attenuates EV promoted invasion

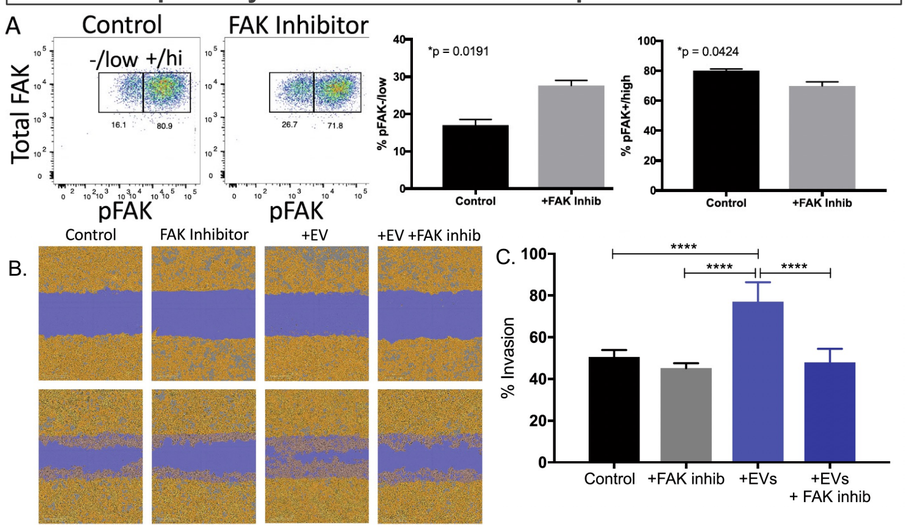


Figure 4: FAK pathway inhibition attenuates increased invasion after EV treatment. **A.** Incubation with FAK inhibitor decreases phosphorylated FAK protein as detected by flow cytometry. **B.** Representative images of cell densities (yellow) overlaid with initial scratch wounds (purple). **C.** The average percent invasion of 4 replicate wells containing DCIS cells treated with EVs ±3μM FAK inhibitor

Conclusions

Our results suggest that circulating EVs from YWBC patients contain biologically relevant cargo that alter the behavior of cancer cells and may influence disease progression. Further, these EVs contain a unique set of proteins that could potentially serve as cancer biomarkers, and others that may be potential targets for individualized cancer treatment.

References

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