**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**

**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 transport biologically significant molecules such as proteins and nucleic acids and contribute to cancer progression and metastases. They may also serve as biomarkers of various disease states and represent important therapeutic targets. Breast cancer EVs have the potential to change the behavior of other cells in their microenvironment. However, the proteomic content of EVs isolated from young women's breast cancer patients and the mechanisms underlying their effects on tumor cell behavior have not yet been reported.

**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 evaluated the gene expression changes in tumor cells after incubation with EVs.

**Results**: We found that treatment with EVs from both invasive breast cancer cell lines and plasma of YWBC patients altered the invasive properties of non-invasive breast cancer cells. Proteomics identified differences in EV content from YWBC patients and healthy donors that correlated with this functionality. Further, we identified gene expression changes in non-invasive breast cancer cells after treatment with EVs that implicate the Focal Adhesion Kinase (FAK) signaling pathway as a potential targetable pathway affected by breast cancer-derived EVs.

**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.