Impact of Tumor and Adipose-Derived EVs on Metabolic Regulation and Metastatic Progression in Triple-Negative Breast Cancer from Obese and Nonobese Mice

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Background: Women with obesity have an increased risk of developing triple-negative breast cancer (TNBC) and its progression to metastasis. Extracellular vesicles (EVs) are tissue-secreted nanoparticles that carry proteins, lipids, and nucleotides facilitating cell communication. Thus, understanding how EVs control the function of target cells could shed light on the link between obesity, TNBC, and metastasis. Previously, we conducted proteomic analyses of EVs isolated from the mammary tumors and adipose tissue of obese and non-obese mice, identifying the presence of pyruvate carboxylase (PC). This anaplerotic enzyme in cancer promotes angiogenesis, immune evasion, and metastasis. We hypothesize that tumor and adipose-derived EVs increase the expression of PC and genes involved in the epithelial-to-mesenchymal transition (EMT) in non-metastatic mammary cancer cells. **Methods**: We purified EVs from visceral adipose tissue and mammary tumors of obese and non-obese mice using differential centrifugation in combination with a flotation iodixanol density gradient. Subsequently, M-wnt and E-wnt murine cell lines were co-cultured with tumor or adipose EVs for 24 hours. We used confocal microscopy to confirm the internalization of the EVs. The PC levels in the cells treated with EVs were determined by western blot, and qPCR was used to measure the expression of *PCx* and *Snail1* and *Snail2*. **Results**: We observed the internalization of EVs after 24 hours of culture. Most of the EVs were intact. We measured no significant changes at the protein and transcript levels of PC in M-Wnt and E-Wnt cells co-cultured during 24 or 48 hours with tumor or adipose-derived EVs from obese and non-obese mice. The expression of the EMT markers, *Actb*, *Snail1,* and *Snail2* were not significant. To better understand how EVs influence metastatic progression, future analysis will investigate the effects of EVs on other EMT markers, including E-cadherin and N-cadherin, after a longer period of co-culture.