The role of breast cancer exosomes in the preparation of the pre-metastatic niche: an *in silico* approach.

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Breast cancer is the most common type of cancer in women and its mortality is related to the development of metastasis. Metastasis is a multistep process in which tumor cells detach from the tumor bulk, pass through the endothelium, and reach the circulatory system leading to the extravasation to a site suitable for the growth of a secondary tumor – the pre-metastatic niche (PMN). Recent studies are unraveling the role of extracellular vesicles (EVs) shed by cancer cells in the preparation of the PMN, which can lead to an increased attachment of circulating cancer cells into the endothelium and, thus, facilitating its extravasation. However, it is not clear the molecular mechanism involved in this process. Therefore, our main goal is to elucidate the main proteins involved in this process by an *in silico* approach. We investigated molecules involved in the interaction between exosomes shed by the triple-negative breast cancer cell line MDA-MB-231 (exoMDA) and human umbilical vein endothelial cell line HUVEC. We re-analyzed published datasets containing proteomics data of and HUVEC. We also re-analyzed a RNAseq dataset of HUVEC treated for 72h with exoMDA using GEO2R. To identify the main players in each condition, we investigated the differentially expressed genes (DEGs) through gene ontology enrichment (GO) and protein-protein interactions (PPI). Networks were obtained using STRING database through Cytoscape *software*, evaluating sub-networks using the MCODE, and identifying the main nodes using Cytohubba. Prior to treatment, exoMDA had 298 DEG and HUVEC, 1949. Both samples shared 222 genes in common. The PPI networks of those three sets of genes were obtained and merged, resulting in a network with 1946 nodes and 12792 edges. Then, we divided the main network into 12 subnetworks enriched with GO terms such as hemostasis, apoptosis, membrane trafficking, and PI3K/AKT pathway. The main nodes were TUBB4B, PIK3R1, PIK3CA, ARF3/1, HMGA2, EP400, RAB7A, and ITGA4. After treatment with exoMDA, we observed 115 upregulated and 139 downregulated genes in HUVEC. The resulting PPI network with the upregulated genes had 80 nodes and 57 edges, generating a single subnetwork and enriched with GO terms such as focal adhesion, PI3K/AKT and MAPK

signaling. The main nodes were PROM1, FLT4, and ITGA4. ITGA4 remained expressed in both conditions, establishing itself as one of the main nodes in both networks and interacting with exosomal RAP1A. In conclusion, we observed that exoMDA could be affecting different steps of the development of a PMN by interacting with endothelial cells, leading to changes in the molecular signature and the behavior of those cells. Moreover, the activation of PI3K/AKT signaling and the presence of ITGA4 could be playing a major role throughout the interaction between exoMDA and HUVEC. Our perspective is to further study those molecules through an *in vitro* approach.