The Role of Exosomes in Breast Cancer Cell Redirection

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One of eight women in the United States will be diagnosed with breast cancer in her lifetime. But of the estimated 250,000 women who are newly diagnosed this year, the lives of over 40,000 of these women will be lost. Despite recent improvements in survival rates in the last few decades, there is still much room for the development of methods for breast cancer diagnosis and treatment. A novel approach to further understand tumor progression would allow for new targets and treatments to emerge. Previous studies have shown that normal mouse mammary niches can guide development and differentiation of resident mammary cells, cells of non-mammary origin (such as tumor derived cells) and surrounding tissue. When non-mammary tumor-derived cells are placed in developing mammary stem cell niches they have been seen to proliferate and be “redirected” i.e lose their cancer-proliferating capacity and assume mammary epithelial phenotype/normal mammary epithelial gene expression. This study focuses on the identification of the exosome cargos/pathways involved in the intercellular communication interactions controlling cancer cell redirection for potential therapeutic targeting.

Mammary epithelial cells (MECs) were grown in media supplemented with exosome-depleted fetal bovine serum (FBS). The exosomes used in this study were collected from 2 and 3 day cultures (MCF10A, MCF12A, HCC1954, SKBR3, BT474) using two primary exosome isolation methods: commercially available isolation kits (Thermofischer-Total Exosome Isolation Reagent (from cell culture media)) and via a novel spin-down tip method using a capillary-channeled polymer (C-CP) fiber phase in a hydrophobic interaction workflow. Collected exosomes were filtered with 0.5 micron sterile filters and analyzed for CD9, CD63, and CD81 by Western blotting and 24hr sandwich ELISAs. MECs (MCF10A, MCF12A) were also transfected with pCD81-GFP to confirm if stable transfection was possible for future cell uptake studies.

Breast cancer cell lines (SKBR3,HCC1954, BT474) and normal mammary cells (MCF12A, MCF10A) displayed regular activity after being grown to 70-80% confluency over 2 and 3 days. The isolation of exosomes using the commercial kit methods produced low quantity and quality populations of exosomes, causing Western blot analyses to be a challenge. To counter that issue, the C-CP spin down tip isolation method and ELISAs were used to isolate exosomes and identify the presence of key tetraspanins (CD9, CD63, CD81) involved in exosome activity. In terms of transfection, pCD81-GFP transfection was seen to be stable for up to 48 hours. The presence of the CD9, CD63, and CD81 proteins in the exosomes recovered confirmed the endosomal origin of secreted vesicles. In the future, we will assess the exosomal contents and their impact on breast cancer cells.

Acknowledgments: This research was financially supported by South Carolina Idea Networks of Biomedical Research Excellence (SC INBRE).