THE ROLE OF EXTRACELLULAR VESICLES IN BREAST CANCER: A POTENTIAL ANTITUMOR PEPTIDE

A Distinguished Lecture

By

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Abstract: How breast cancer cells and primary mammary epithelial cells interact and communicate with each other to promote tumorigenesis and how to prevent tumor metastasis has long been a concern of researchers. Cancer cells secrete exosomes containing proteins and RNA. These factors can influence tumor development by directly targeting cancer cells and tumor stroma. We developed a peptide derived from the Secretion Modification Region (SMR) of HIV-1 Nef protein that was modified with PEG on the N-terminus, and with a Clusterin (Clu)-binding peptide on the C-terminus. Attachment of PEG to the SMR peptide, termed PEGylation, offers improved water solubility and stability as well as reduced clearance through the kidneys, leading to a longer circulation time. The 12-mer Clu-binding peptide plays multiple roles in tumor development and metastasis. The Clu peptide can be detected by antibody *in vivo*, thus having the potential to be used to monitor tumor status and treatment efficacy in animal studies and eventually in cancer patients. MCF-7 and MDA-MB-231 breast tumor cells were treated with PEG-SMR-Clu peptide alone, and in combination with paclitaxel and cisplatin. Cell proliferation and viability were determined via cell cycle analysis using Cellometer imaging cytometry, Annexin V, and MTT assays. The effects of the PEG-SMR-Clu peptide on tumor exosome release were determined by testing isolated exosome fractions, for (i) expression of CD63 and Alix proteins by Western blotting, (ii) NanoSight nanoparticle tracking analysis (NTA 10) to measure exosomes size and concentration, and (iii) measurement of acetylcholinesterase (AchE) for exosome specific enzyme activity. PEG-SMRwt-Clu and PEG-SMRwt peptides inhibited the growth of both MCF-7 (estrogen responsive, ER+) and MDA-MD-231 (estrogen non-responsive, ER-) human breast cancer cells in a dose and time-dependent manner, without inducing cytotoxic effects. The SMRwt peptide, combined with paclitaxel, induced G2/M phase cell cycle arrest on MCF-7 and MDA-MB-231 cells but did not promote apoptosis. PEG-SMRwt-Clu peptide treatment blocked exosome release from both MCF-7 and MDA-MB-231 cells. The peptide effect was blocked by knockdown of the chaperone protein mortalin by either antibody or siRNA. PEG-SMRwt-CLU peptides inhibited the growth of human breast cancer cells and blocked tumor exosome release in vitro. The peptide alone did not increase cytotoxicity or apoptosis induction, but did cause cell cycle G2/M phase arrest in both estrogen responsive and non-responsive breast cancer cells. These data suggest a potential therapeutic value of SMR to prevent breast cancer metastasis and as an adjuvant for the chemotherapeutic treatment of human breast cancer.