Breast tumors stem cells have increased microtentacles that can be targeted therapeutically with curcumin.

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Breast cancer is the 2nd leading cause of cancer-related death among women; however, the majority of deaths arise as complications from metastasis, rather than the primary tumor. The cancer stem cell (CSC) hypothesis provides an explanation for the limited success of current therapies for metastatic breast cancer. CSCs are defined as a subpopulation of tumor-initiating cells with the stem cell-like characteristics of self-renewal and multipotency. CSCs are known to come into circulation and reach distant tissues, where they may remain cell-cycle arrested, and therefore resistant to current chemotherapies.

Recent work in our lab has shown that circulating tumor cells use dynamic tubulin-based microtentacles (McTNs) to adhere to distant tissues, a critical step in metastasis. McTNs are novel cellular structures formed by epithelial cells when detached from the extracellular matrix and are increased in more metastatic breast cancer cell lines. Given the proposed metastatic efficiency of CSCs, we examined McTN incidence and function in mammary stem cells and breast cancer stem cells. Flow cytometry for the CSC marker CD44 and CD24 showed breast tumor cell lines with increased CSC characteristics display higher McTN frequencies. Given this correlation, CD44 and CD24 immunofluorescence was used to separate human mammary epithelial (HMLE) cells into CSC and non-CSC subpopulations by flow cytometry. Stem-like HMLE cells (CD44hiCD24lo) have increased McTN levels compared to non-CSC cells (CD44loCD24hi) from the same HMLE cell line. The CSC subpopulation also demonstrated increased cytoskeletal modifications that promote McTN formation in suspended stem-like HMLEs. The increased McTNs in stem-like HMLEs promote faster initial reattachment of suspended cells that is inhibited by the tubulin-depolymerizing agent, colchicine, confirming a functional role for McTNs in reattachment. Moreover, five cell contact microscopy demonstrates that McTNs participate in the structure of breast stem cell mammospheres, encircling adjacent cells and penetrating between cell-cell junctions. McTNs can be reduced by the CSC targeting drug curcumin. These studies show that McTNs contribute to the metastatic potential of breast CSCs as well as the ability of mammary stem cells to form multicellular mammospheres. We anticipate that this work will clarify the molecular mechanisms underlying tubulin alterations in breast cancer stem cells as well as the ability of mammary stem cells to form multicellular mammospheres. We anticipate that this work will clarify the molecular mechanisms underlying tubulin alterations in breast cancer stem cells as well as the ability of mammary stem cells to form multicellular mammospheres.

INTRODUCTION

Human mammary epithelial cells and other transformed mammary cell lines produce unique protrusions called the plasma membrane when detached from extracellular matrix.

RESULTS

Stem-like mammary epithelial cells have increased microtentacles that can be targeted therapeutically with curcumin.

1. Examine the incidence and function of microtentacles in breast stem-like subpopulations.
2. Define the role of detyrosinated tubulin and vimentin in the generation of microtentacles in breast stem-like subpopulations.

Figure 1: Epithelial cells produce long, dynamic, tubulin-based protrusions upon detachment termed microtentacles (McTNs). (A) Treatment of detached cells with the actin depolymerizing agent, Latrunculin-A (LA) promotes the detachment process. (B) Immunofluorescence microscopy of breast cancer cells labeled with a WGA-Alex488 displays the complexity and curvature of these protrusions. (C) Human mammary epithelial cells exhibit tubulin-based membrane ruffling and protrusion-like structures upon detachment. (D) Vimentin and tubulin, two cytoskeletal proteins, are co-localized in these microtentacles.

Table 1: McTNs correlate with increased stemness across a panel of breast cancer cell lines. A panel of breast cancer cell lines were compared for their incidence and metastatic potential, microtentacle levels, and subpopulations of stem-like (CD44+CD24+) and non-stem-like cells (CD44+CD24-). Invasiveness, metastatic capability, and microtentacle levels increased with increasing stem cell character.

Figure 2: Imbalances in cytoskeletal physical forces increase cell deformability and McTNs. (1) In attached epithelial cells, the outward force of microtubules originating from the cell center (green) counteracts the contraction of the cortical actin cytoskeleton (red). (2) Upon detachment, many epithelial cells die by anovus. In some cells, mild relaxation occurs in the actin cortex and microtubules are processed to a stabilized detyrosinated form that promotes microtentacle extension. (3) In some cancer cells, the physical forces in tumor cells are dysregulated. The cytoskeletal perturbations that produce epidermal micropapillae-like structures likely arise from a combination of persistent weakening of the actin cortex (leading to increased deformability) and increased extension and stability of the actin-myosin-I and microtubule association with stabilizing MAPs or vimentin.

Figure 3: Stem-like mammary epithelial cells have increased microtentacles and microtentacle-promoting cytoskeletal alterations. (A) HMLE cells have distinct subpopulations of stem-like (CD44hiCD24lo) and non-stem-like (CD44loCD24hi) cells. Phase contrast images demarcate HMLE subpopulations where non-stem-like HMLEs display increased microtentacles (black arrows) compared to non-stem-like HMLEs. (B) Stem-like subpopulations of HMLEs display significantly higher microtentacle frequencies than non-stem-like subpopulations. Columns, mean for three blinded experiments where at least 100 HMLE-stained cells were counted, representative of three independent experiments; bars, SD (P ≤ 0.0005, t test, black asterisks). Representative western blot analysis of HMLE subpopulations shows that stem-like HMLEs have increased vimentin and detyrosinated tubulin (Glu-tub) whereas total tubulin and GAPDH are comparable.

Figure 4: Stem-like mammary epithelial cells have microtentacle-promoting cytoskeletal alterations. (A) RNA was isolated from HMLE subpopulations using TRIzol reagent and reverse transcribed into cDNA. qPCR analysis reveals increased levels of Glu-tubulin (Glu-tub) and a loss of vimentin protein and organization (vimentin) (B) in stem-like subpopulations of HMLEs compared to non-stem-like subpopulations. (C) Western blot analysis of HMLE subpopulations with antibodies against p-tubulin and vimentin reveals the presence of Glu-tubulin (Glu-tub) and vimentin in stem-like HMLE cell lines but not in the non-stem-like subpopulation (h). HMLB was used to visualize the nuclei (a).

Figure 5: Mammary stem-like cells have increased tubulin-dependent initial reattachment from suspension. (A) Bt549s and MDA-231 cells display significantly higher microtentacle frequencies than MCF10A and MCF7. (B) Immunofluorescence microscopy of Bt549s and MDA-231 cells reveals increased microtentacles (black arrows) in the stem-like cell population compared to the non-stem-like cell population. (C) qPCR analysis of Bt549 and MDA-231 subpopulations shows that stem-like Bt549s and MDA-231s have increased vimentin expression compared to non-stem-like Bt549s and MDA-231s. (D) Western blot analysis of Bt549 and MDA-231 subpopulations reveals increased levels of Glu-tubulin (Glu-tub) in stem-like Bt549s and MDA-231s compared to non-stem-like Bt549s and MDA-231s. (E) Representative western blot analysis of Bt549 subpopulations shows that stem-like Bt549s have increased vimentin and detyrosinated tubulin (Glu-tub) whereas total tubulin and GAPDH are comparable.

Figure 6: Microtentacles Persist in Mammospheres. 1. Examine the incidence and function of microtentacles in breast stem-like subpopulations. 2. Define the role of detyrosinated tubulin and vimentin in the generation of microtentacles in breast stem-like subpopulations.

Figure 7: Microtentacles Persist in Mammospheres. 1. Examine the incidence and function of microtentacles in breast stem-like subpopulations. 2. Define the role of detyrosinated tubulin and vimentin in the generation of microtentacles in breast stem-like subpopulations.

Figure 8: Targeting circulating CSCs to reduce metastasis. The heterogeneous cells of the primary tumor, while cells are able to survive to conditions of the circulatory system. Breast cancer stem cells (CSCs, in yellow) are primed to undergo successful metastasis and eventual outgrowth due to their stem cell properties of self-renewal, multipotency, and drug resistance. These cells are also more deformable, and produce greater numbers of METs, which facilitate initial reattachment from suspension to the capillary endothelium, providing death via fragmentation in narrow capillaries. Identifying the mechanisms by which CSCs increase McTN formation will aid in the design of novel therapeutics to target circulating cancer stem cells.