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TITLE: Are Breast Tumor Stem Cells Responsible for Metastasis and Angiogenesis?

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14. ABSTRACT Despite advances in the early detection and treatment of breast cancer, the mortality rate for 20% of patients with recurrences and/or metastases is nearly 100% (1). Thus, it is critical to understand the mechanism of breast cancer metastasis to allow for better therapies targeted at the cellular culprits of metastasis. The current dogma of metastasis is that most primary tumor cells have low metastatic potential, but rare cells, less than one in ten million, within large primary tumors acquire metastatic capacity through somatic mutation. The metastatic phenotype includes the ability to disseminate from the primary tumor, survive in blood or lymphatic circulation, invade distant tissues and establish macroscopic metastatic nodules. This dogma is primarily supported by animal models in which highly metastatic clones can develop from poorly metastatic cell lines if the process is facilitated by the isolation of metastatic nodules, expansion of the cells <i>in vitro</i> , and injection of these selected cells into recipient mice. However, no direct evidence of this genetic selection model has been documented in human tumors. A recent report demonstrated that a subpopulation of breast tumor cells (CD44+/CD24-(low)/Lineage-) isolated from breast cancer patient samples, even as few as 200 cells, were able to give rise to bulky tumors, greater than 1 cm in diameter, in NOD/SCID mice. Moreover, this discrete cell population has the ability to proliferate extensively, and to give rise to diverse and more differentiated cell types with reduced developmental or proliferative potential suggesting that these highly tumorigenic CD44+/CD24-(low)/Lineage- cells may, indeed, be breast tumor stem cells. With this information, we propose an alternate model of metastasis and hypothesize that the breast tumor stem cells are the subpopulation of cells that are present in the heterogeneous primary breast tumor and possess the unique properties of an angiogenic and metastatic phenotype.					
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Introduction:

Despite advances in the early detection and treatment of breast cancer, the mortality rate for 20% of patients with recurrences and/or metastases is nearly 100% (1). Thus, it is critical to understand the mechanism of breast cancer metastasis to allow for better therapies targeted at the cellular culprits of metastasis. The current dogma of metastasis is that most primary tumor cells have low metastatic potential, but rare cells, less than one in ten million, within large primary tumors acquire metastatic capacity through somatic mutation (2). The metastatic phenotype includes the ability to disseminate from the primary tumor, survive in blood or lymphatic circulation, invade distant tissues and establish macroscopic metastatic nodules. This dogma is primarily supported by animal models in which highly metastatic clones can develop from poorly metastatic cell lines if the process is facilitated by the isolation of metastatic nodules, expansion of the cells *in vitro*, and injection of these selected cells into recipient mice (3). However, no direct evidence of this genetic selection model has been documented in human tumors. A recent report demonstrated that a subpopulation of breast tumor cells (CD44+/CD24-(low)/Lineage-) isolated from breast cancer patient samples, even as few as 200 cells, were able to give rise to bulky tumors, greater than 1 cm in diameter, in NOD/SCID mice (4). Moreover, this discrete cell population has the ability to proliferate extensively, and to give rise to diverse and more differentiated cell types with reduced developmental or proliferative potential suggesting that these highly tumorigenic CD44+/CD24-(low)/Lineage- cells may, indeed, be breast tumor stem cells (4). With this information, we propose an alternate model of metastasis and hypothesize that the breast tumor stem cells are the subpopulation of cells that are present in the heterogeneous primary breast tumor and possess the unique properties of an angiogenic and metastatic phenotype. This proposal will address the importance of tumor stem cells in breast cancer metastasis using an appropriate animal model. As a corollary, it may suggest therapies that selectively target the breast tumor stem cell population leading to abrogation of clinical metastasis, a recalcitrant challenge in breast cancer.

Body:

Specific Aim 1:

1. Determine the *in vitro* angiogenic potential of unsorted breast tumor cells, sorted putative stem tumor cells, and established breast cancer cell lines using the rat aortic ring, endothelial cell vessel formation, and endothelial cell migration assay (Months 1-4).

2. Determine the in vitro invasion and motile potential of unsorted breast tumor cells, sorted putative stem tumor cells, and established breast cancer cell lines (Months 3-6).

Specific Aim 2:

1. Determine the in vivo metastasis potential of unsorted breast tumor cells and sorted putative stem tumor cells using the tail vein injection pulmonary colonization and intracardiac injection bone metastasis models (Months 6-9).
2. Isolate tumor cells from the metastases, resort for putative breast tumor stem cells, and serially transplant into secondary recipient mice. Determine the in vivo metastatic potential of serially transplanted putative breast tumor cells (Months 7-12).
3. Characterize the heterogeneity of the tumor cells from the metastases using flow cytometry (Months 9-12).

I was granted a no-cost time extension for this concept award for a period of one year. This extension was needed as we encountered technical difficulties that hindered the progress of this grant in a timely manner. We had difficulty finding the appropriate combination of cell surface markers to identify the breast tumor stem cell population from tumors in Her2/neu transgenic mice. We have made some progress in identifying the cell surface markers to use and are now actively working on the specific aims as proposed for this concept award.

Key Research Accomplishments and Reportable Outcomes:

Experiments are still ongoing and thus, we do not have reportable outcomes and key research accomplishments to report at this time.

Conclusions:

We do not have conclusions to report at this time.

References

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Personnel Paid From This Concept Award

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