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TITLE: Cellular Plasticity of Epithelial Cells-Cause of Metastasis

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14. ABSTRACT

Metastasis is a major factor contributing to casualties in cancer patients. In this award, we sought to address critical questions as to how the rare metastatic epithelial cells can find and contact the much rarer angiogenic endothelial cells, or how a new angiogeneic network is setup. We present a novel concept that cancer epithelial cells, possibly of stem cell origin, have inherent cellular plasticity and can differentiate into endothelial cells and form microvessels that serve as a conduit for entry of epithelial cells To test this hypothesis we marked metastatic breast cancer cells with GFP and looked for expression of endothelial markers in the green epithelial cells.

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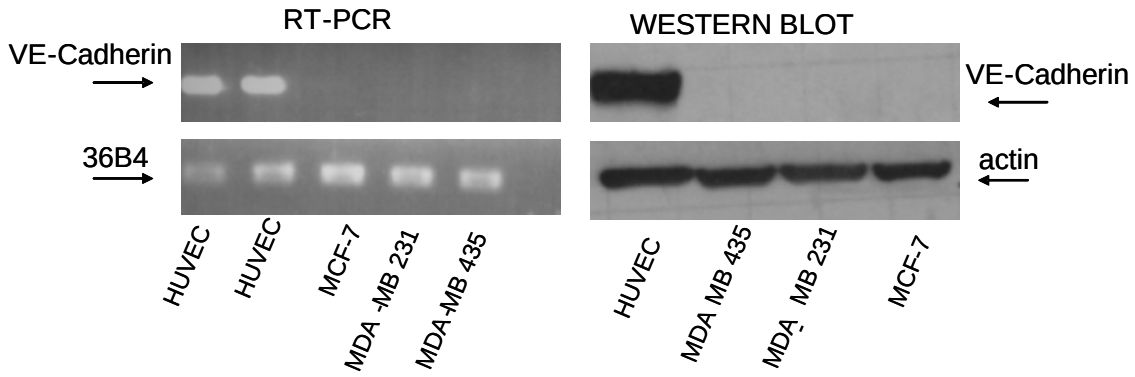
Concept award:
Pricipal Investigator: Saraswati Sukumar, PhD

Metastasis is a major factor contributing to casualties in cancer patients. However, critical questions as to how the rare metastatic epithelial cells can find and contact the much rarer angiogenic endothelial cells, or how a new angiogeneic network is setup, are rarely addressed. We present a novel concept that cancer epithelial cells, possibly of stem cell origin, have inherent cellular plasticity and can differentiate into endothelial cells and form microvessels that serve as a conduit for entry of epithelial cells into the circulation.
To test this hypothesis we undertook the following steps.

Statement of Work:
These experiments will address the question of epithelial cell plasticity and function in the breast cancer metastatic process.
Months 1-3:
Task 1: Breast cancer cell lines, MDAMB231 and MDAMB468 will be stably transfected with enhanced green fluorescence protein (GFP)-marker, selected using G418 and stable clones will be generated that have strong expression of EGFP.

To test this hypothesis, we first needed to label the epithelial cancer cells with GFP and inject the cells into mouse mammary glands. Thereby, we could track these cells and check whether these cells can express the endothelial specific markers *in vivo*. Thus, we established stable pool and single clones of GFP-labeled non-metastatic MCF-7 cells, metastatic MDA-MB 231 and MDA-MB 435 cells.
Since our goal is to demonstrate epithelial to endothelial transition in vivo, we first used RT-PCR and western blot to ensure that no endothelial specific marker, VE-Cadherin, was expressed in these cells. (see figure below)



Months 4-6
Task 2: A million tumor cells from a stable clone with strong EGFP expression of the two cell lines will be injected into groups of 6 SCID mice to grow as tumors in the fat pad. Two- three weeks later, when the tumors are about 10 mm in diameter, the tumors will be excised.

These cells were injected into nude mice both by intramammary gland injection and intracardiac injection. After 8 weeks, both in situ and metastatic tumors to lung and liver were recovered and fixed with formalin.

Months 6-8
Task 3: Immunostaining of the tumor fixed in formalin, or preserved as frozen tissues will be performed. Anti-GFP antibody, and endothelial specific antibodies (PH12, von Willebrand factor, VE-cadherin) will be used to study expression in blood vessels and tumor cells.

Tumors recovered from the mice have been paraffin embedded. Immunofluorescent staining on these samples will be used to check whether these green cancer epithelial cells will express VE-Cadherin. If so, we will also check whether VE-Cadherin expression will be different between non-metastatic and metastatic cells.

Months 5-8:
Task 4: The tumor pieces from Task 3 will be serially passaged three times as xenografts in the SCID mouse mammary fat pad. We will perform staining for markers of endothelium, to see if the tumor cell populations have an increasing proportion of cells expressing endothelial markers in later passages.

Tumor pieces have been transplanted for serial passage. Further passage tumors will be collected for examination of endothelial markers.

Months 8-12
Task 4: We will construct a vector with expression of Herpes thymidine kinase (HTK), a drug susceptibility gene, under the control of endothelial specific promoter, VE-cadherin. This construct will be transfected into MDAMB231 and stable clones will be derived by G418 selection.

Task 5: Cells will be grown as tumors in the mouse mammary fat pad (as in task 1), and treated with acyclovir or ganciclovir (2X a day for 5 days). Cells with endothelial transformation alone will die. If this epithelial to endothelial transformation is critical for metastasis, mice treated with drug will show significantly lower metastasis than the control.

Task 6: Evaluate results. Plan new detailed experiments if the results reflect the hypothesis.

Tasks 5 and 6 will be undertaken as soon as clones of MDAMB 231 cells are ready.

Key Research Accomplishments:

1: Established cell lines expressing GFP

2: Xenograft asssays were done.

3. Passage of xenograft assays ongoing.

Reportable Outcomes

Lianfeng Han's presentation at Era of Hope conference of abstract entitled "Cellular Plasticity of

Epithelial Cells-Cause of Metastasis”.

Conclusions

Work is ongoing and conclusions will be drawn after data has been analyzed.

References

None

Appendices

None