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TITLE: Molecular Tracking of Proteolysis During Breast Cancer Cell Extravasation: Blockage of Therapeutic Inhibitors

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Elucidating the proteolytic effectors responsible for focal proteolysis at the cellular/pericellular matrix interface during metastasis is an on-going challenge. We have begun to dissect the molecular dynamics of proteolysis required for tumor cell transendothelial migration. MDA-MB-231 (metastatic) cells are far more efficient at TEM compared to MCF-7 (non-metastatic) cells. MMP-2, MMP-9, MT1-MMP and TIMP-2 appear active participants during breast cancer TEM. Natural (TIMP-1, TIMP-2) as well as synthetic MMP inhibitors significantly reduced TEM of both MCF-7 and MDA-MB-231 cells. Serine inhibitors also significantly reduce TEM. Furin is detectable intracellularly as well as at the cell surface of MDA-MB-231 cells. Future experiments will focus on identification of molecules that are necessary for complex formation of the MMPs at the cell-matrix interface during TEM.

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Table of Contents

Introduction:

Elucidating the proteolytic effectors responsible for focal proteolysis at the cellular/pericellular matrix interface during metastasis is an on-going challenge. Here we dissect the molecular dynamics of proteolysis required for tumor cell transendothelial migration (TEM).

Body:

We have completed the determination of the MMP profile for MCF-7 and MDA-MB-231 breast cancer cells as well as human lung microvascular endothelial cells by Zymography. This has been performed on individual cell lines and TEM co-cultures. Verification of key MMP and TIMP expression in these cells has been further obtained at the protein level by western blotting. Both MMP-2 and MMP-9 are present, and among TIMPs, TIMP-2 is an important molecule. MT1-MMP is present only in MDA-MB-231cells suggesting the involvement of trimolecular complex. We are currently assessing the quality of immunofluorescence staining in TEM cultures using antibodies from a number of sources (commercial and those obtained as gifts from investigators). While MMP-2, MMP-9 and MT1-MMP antibodies from Oncogene and Chemicon have worked well, an antibody from Dr. W. Garten (Marburb University, Germany) has been instrumental for detecting furin in our TEM system. We have obtained data on biological and synthetic MMP-inhibitors as well as serine inhibitor mediated reduction of breast cancer cell transmigration. MMP inhibitors, serine inhibitors, TIMP-1 and TIMP-2 show a statistically significant reduction in TEM of breast cancer cells. Surprisingly, TIMP-1 and TIMP-2 did not exert additive inhibitory effect. We also have optimized conditions for assessing MMP-based fluorogenic substrate digestion in TEM cultures by confocal microscopy.

Key research Accomplishments:

- Determination of MMP profile in isolated and TEM cultures
- Validation of identified MMPs by western blotting
- Identification of antibodies for good quality immunofluorescence signal
- Inhibition of breast cancer cell TEM by synthetic inhibitors
- Optimization for MMP-mediated substrate digestion in TEM assay

Reportable Outcomes:

We are in the process of writing our first manuscript supported by this grant

Conclusions:

Optimization of conditions for non-metastatic (MCF-7) and metastatic (MDA-MB-231) breast cancer cells in our TEM assay, at several levels as described above, forms the foundation for future experiments. We have narrowed the MMPs implicated in this process, identified the reagents to be used, and developed a protocol for quantifying substrate digestion. This is in keeping with the milestones projected.