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TAFIa Instability and its Role in Breast Cancer Metastasis

Importance: Thrombin activatable fibrinolysis inhibitor (TAFI) is a plasma zymogen that can be activated to its active form TAFIa by thrombin in complex with thrombomodulin¹. TAFIa has basic carboxypeptidase activity and is able to cleave carboxyl terminal lysine and arginine residues on various protein and peptide substrates, including cell-surface receptors for plasminogen^{1,2}. Research in our laboratory has revealed that TAFIa plays a key role in reducing tumour invasion and migration in breast cancer⁶. These studies aim to provide insight into potential strategies to therapeutically activate TAFI in order to prevent tumour cell invasion and migration in breast cancer.

Existing State of Knowledge: TAFIa plays an important role in inhibiting pericellular plasminogen activation at the cell surface by cleaving carboxyl terminal lysine residues on plasminogen receptors, thus preventing the formation of plasmin⁸. Plasmin, along with matrix metalloproteases, degrades the extracellular matrix (ECM) to facilitate tumour cell invasion and metastasis as well as angiogenesis in breast cancer and to liberate ECM-associated growth factors^{6,8}. Interestingly, TAFIa is intrinsically unstable with a half-life of approximately eight to fifteen minutes at body temperature⁷; more stable variants of TAFIa are correspondingly more effective in inhibiting fibrinolysis. It is unknown whether TAFIa instability similarly regulates its antimetastatic potential in breast cancer. In order to address this, recombinant variants of TAFI which differ in their intrinsic stability will be studied.

Research Question: We hypothesize that TAFIa variants with an increased half-life will more effectively inhibit plasminogen activation and metastatic behaviour of breast cancer cells.

Methodology: Recombinant variants of TAFI which differ in their intrinsic stability: Thr325 (half-life of 8 minutes), Ile325 (15 minutes)³, R302Q (1.5 minutes)³ and a stable form of TAFIa consisting of five point mutations (1140 minutes)³⁻⁵ were constructed using site-directed mutagenesis. The Thr/Ile325 variants represent a common polymorphism observed in the human population³. Using triple negative MDA-MB 231 and SUM149 human breast cancer cell lines – which are characterized as highly invasive and metastatic – cell invasion, cell migration, extracellular proteolysis and cell proliferation assays will be performed utilizing the purified recombinant variants of TAFI differing in intrinsic stability.

Findings: Preliminary experiments suggest that treatment with the activated stable form of TAFI decreases metastasis in breast cancer. This is the first direct evidence for an antimetastatic role, as our previous experiments used a potent inhibitor of TAFIa to show that inhibition of this enzyme stimulated metastasis. Further experimentation using activated Thr325, Ile325, and R302Q variants will be conducted.

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