Focal Adhesion Kinase Signaling in Transendothelial Migration of AU-565 and MDA-MB-231 Breast Cancer Cells

by

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ABSTRACT

Cancer occurs when hyperproliferating cells have acquired the ability to invade tissues. Metastasis involves tumor cell invasion of the stroma surrounding the primary tumor, transport throughout the body by means of the vasculature, and escape from the vasculature and subsequent proliferation of a secondary tumor. This thesis investigates extravasation, which involves migration of tumor cells through the endothelial lining of blood vessels. Extravasation can be monitored in real-time using electric cell-substrate impedance sensing (ECIS). ECIS measures endothelial monolayer resistance, which drops when exposed to metastatic cancer cells.

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase and scaffold protein that promotes turnover of focal contacts. Focal contacts are dynamic structures that mediate cell adhesion. $FAK^{-/-}$ fibroblasts show motility defects, including decreased 2-dimensional migration and delayed invasion. Phosphorylation of specific FAK tyrosine residues (397, 861, and 925) triggers distinct signaling pathways that result in migration and invasion through extracellular matrix. We hypothesized that FAK participates in transendothelial migration of breast cancer cells, through similar signaling mechanisms.

Inhibiting FAK using siRNA or FAK-related non-kinase (FRNK) results in decreased or delayed transendothelial migration of AU-565 breast cancer cells, demonstrating that FAK participates in this process. However, over-expression of FAK does not promote transendothelial migration of either AU-565 or MDA-MB-231 cells. FAK mutants provide information about more specific signaling events involved in transendothelial migration. AU-565 and MDA-MB-231 cells expressing Phe397 FAK show delayed transendothelial migration, demonstrating the involvement of the FAK autophosphorylation site. MDA-MB-231 cells but not AU-565 cells expressing Phe861 FAK (Cas binding site mutant) also exhibit delayed transendothelial migration. Both AU-565 and MDA-MB-21 cells expressing Phe925 FAK (Grb2 binding site mutant) show no change in transendothelial migration compared to untreated cancer cells.

Elucidation of signaling pathways involved in transendothelial migration, which vary from those involved in 2-dimensional cell migration, will provide targets for chemotherapeutic drug design.