

CXCL7-MEDIATED STIMULATION OF THE LYMPHANGIOGENIC FACTOR VEGF-C AND HEPARANASE ACTIVITY IN HUMAN BREAST CANCER CELLS

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Introduction: Chemokines are increasingly implicated in breast cancer. They may modify breast cancer cells and the surrounding matrix to facilitate metastasis. Furthermore, they are recognized angiogenic factors. Increased expression of the lymphangiogenesis factor VEGF-C and a possible matrix modifying enzyme called heparanase have been correlated with progressive disease in certain cancers. One member, CXCL7, has dual functions of heparin-binding and ligand to a G-protein linked receptor, CXCR2, found on endothelial cells. Increased CXCL7 expression has been associated with the invasive phenotype in breast cancer. The purpose of this study was to determine the effect of CXCL7 on lymphangiogenesis, a critical step in metastasis.

Methods: Premalignant MCF10AT breast cells were stably transfected with CXCL7 using LipofectamineTM2000 (Invitrogen). The transfected cells were sorted by flow cytometry using the EGFP antibody for G418 resistant, and subcultured. The conditioned medium was tested for heparanase activity with the Heparan Degrading Enzyme Assay Kit (Takara) where value of heparanase activity was normalized by protein concentration (U/g protein). The CXCL7 MISSION siRNA was used to knockdown heparanase activity while non-targeting siRNA was used as a control. The VEGF-C gene expression was determined by real time PCR and expressed as a difference between the CXCL7 transfected cells and vector transfected cells for analysis.

Results: VEGF-C mRNA expression was significantly higher in CXCL7-transfected MCF10AT breast cells than in vector transfected cells ($P=0.02$). Heparanase expression was significantly decreased in CXCL7-transfected MCF10AT breast cells with CXCL7 siRNA ($P=0.004$).

Conclusions: CXCL7-transfection increases the expression of VEGF-C and secretion of heparanase, both linked to tumor lymphangiogenesis and metastasis.