Recovery of Circulating Tumor Cells in Urothelial Carcinoma Cell Lines Utilizing a Novel Antibody Based Microfluidic Cell Capture Technique

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INTRODUCTION AND OBJECTIVES: Neoadjuvant chemotherapy (NCTx) prior to radical cystectomy increases survival but is slow in gaining recognition. It is important that we identify appropriate patients for NCTx since once patients develop clinical metastases the median survival is only 12-15 months despite 'gold standard' chemotherapeutic regimens. Currently, traditional risk factors such as tumor grade & stage are used to identify patients at highest risk for developing metastases, but at the expense of over treatment in 50-60%. Evaluating the number of circulating tumor cells (CTC) is one way in which we might be able to improve risk-stratifying patients. Current CTC technology utilizes EpCAM (Epithelial Cell Adhesion Molecule) based capture. However, many tumors, including UC cell lines, have lower expression of EpCAM with more invasive phenotypes. We describe a novel microfluidic cell isolation technology utilizing a combination of antibodies in order to improve the capture of UC cell line cells spiked into blood.

METHODS: A cohort of six UC cell lines were selected based on gene expression heat map analysis as being either more epithelial or more mesenchymal-like. FACS testing for different expression characteristics of cell surface antigens for the UC cell led to formulating an antibody mixture of EpCAM plus 5 additional antibodies to improve CTC capture of all cell types. CTC capture rates were then compared utilizing the antibody mixture versus EpCAM alone with the microfluidic channel assay.

RESULTS: Immunostaining demonstrated that 3 of the cell lines stained for vimentin whereas the remainder had cytokeratin staining with significant EpCAM expression. When utilizing the EpCAM only technique, cell lines without EpCAM expression were not captured. However, with the six-antibody mixture, all 6-cell lines achieved near 100% CTC capture rates.

CONCLUSIONS: We describe a novel antibody-based microfluidic system for CTC capture in UC that demonstrated almost complete capture of all UC cells introduced into the device. This represented a dramatic improvement over cell recovery with EpCAM alone. This technology may be beneficial in recovering CTCs from clinical blood samples containing carcinomas with low EpCAM, or CTCs that may have undergone epithelial to mesenchymal transition (EMT). At this time, a clinical trial is underway to identify a threshold value of number of

CTCs which can be used to risk stratify patients who might be candidates for neoadjuvant chemotherapy.

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