Capture of Circulating Tumor Cells Using Heteromultivalently Decorated Microfluidic Surfaces

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Statement of Purpose: Cancer metastasis is associated with significant mortalities [1]. One main mechanism for metastatic spread is through blood circulation and hence the ability to detect pro-metastatic circulating tumor cells (CTCs) from blood samples could provide a means of evaluating metastatic risks. However, CTC-detection is challenging due to extremely low concentrations of CTCs in blood. Currently, the only FDA-approved technology for CTC detection is the CellSearch® system, that utilizes immunomagnetic labeling of CTCs to enable magnetic separation [2]. A few other technologies are currently under research, that utilize either fluid mechanical or ligand-based biochemical means for CTC capture and separation [3]. In this framework we are investigating a microfluidics-based assay that utilizes heteromultivalent peptide brushes decorated on a surface to enable targeted capture of CTCs. Here we present a 'proof-of-concept' study of this approach utilizing peptides identified from metastatic cell-relevant ligand-receptor interactions. Our vision is to create a 'liquid biopsy' technology for evaluation of metastatic risks from blood samples.

Methods: *Peptide Brush Immobilization on Glass Surface and Evaluation of CTC Capture:* Glass slides were coated with avidin and incubated with biotin-peptide conjugates. As our test metric, we selected peptides based on our recent studies regarding interaction of pro-metastatic breast cancer cells with active platelets [4], as well as, based on reports of high expression of Epidermal Growth Factor Receptors (EGFRs) on various pro-metastatic cancer cell lines [5]. Specifically, we used a P-selectin binding peptide (DAEWVDVS, called henceforth as P1) and a β3-integrin binding peptide (cyclo-CNPRGDY(OEt)RC, called henceforth as P2), based on platelet-cancer cell interactions [4], and an EGFR-binding peptide GE11 (YHWYGYTPQNVI, called henceforth as P3) reported in literature [5]. For model cell lines, we used the low metastatic MCF-7 and the pro-metastatic MDA-MB-231 human breast cancer cell lines. The cells were cultured to ~80% confluence, isolated, counted, and resuspended in culture media to be used for each study. The nuclei of the cells were stained blue fluorescent with DAPI to facilitate imaging with fluorescence microscopy and subsequent counting of adherent cells. Surfaces bearing peptide brushes were created by incubating the avidin-coated glass slides with biotinylated P1, P2 or P3, as well as, combinations of 'P1 + P2', 'P2 + P3', 'P1 + P3' and 'P1 + P2 + P3'. The total moles of peptides incubated in each condition were kept constant, and for the combinations the relative peptide ratio was maintained at equimolar. Each type of coated surface was exposed to a flow of DAPI-stained MDA-MB-231 or MCF-7 at a 5 dynes per cm^2 wall shear in a parallel plate flow chamber, and adherent cells were imaged and counted (using Image J®) at 5, 15, and 30 minutes of flow. Slides coated with bovine serum albumin (BSA), exposed to cells were used as control. *Evaluation of CTC capture in presence of blood:* For these studies, we evaluated the binding of each cell type to homomultivalently vs. heteromultivalently peptide-decorated surfaces, using the same set-up as before, with the modification that only $1x10^2$ cells of each type were resuspended in human whole blood, to test the ability of the peptide brushes to capture CTCs in the presence of blood cells. Adherent cells were imaged and analyzed with fluorescence microscopy as before.

Results: Our results show higher binding of the pro-metastatic MDA-MB-231 cells compared to MCF-7 cells, when surfaces presenting P1, P2 or P3 peptides were used. Furthermore, compared to homomultivalent decorations, heteromultivalent combinations of the peptides significantly enhanced the capture of the MDA-MB-231 cells both in total number, as well as, the rate (i.e. more cells captured faster). We believe that this is because of the cooperative interactions of heteromultivalent ligand-receptor mechanisms that enhance the cumulative arrest of cells that overexpress the target receptors. This trend was also observed in the blood-incorporated assays, even though the number of the circulating cancer cells was significantly lower.

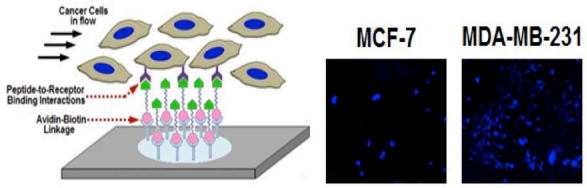

Figure 1. Heteromultivalent decoration of microfluidic surface and representative images of cell capture

Conclusion: The studies demonstrate that feasibility of utilizing heteromultivalent decorations of peptide brushes on microfluidic surfaces for enhanced capture of pro-metastatic CTCs. Further refinement of this approach can lead to an effective CTC capture 'liquid biopsy' technology for prognostic purposes.

References:

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