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14. ABSTRACT

To study circulating prostate cancer cell adhesion behavior and the contribution to distant metastasis of prostate cancer, we applied a dynamic flow-based E-selectin+SDF-1 coated micro-channel model to isolate and characterize a subpopulation of adhering prostate cancer cells. We found a positive correlation of cells' aggressiveness with their adhesion/rolling ability. By using this device we then sorted prostate cancer cell lines (PC3 and DU-145) into rolling and floating populations, and tested for their behavior *in vitro* and *in vivo*. Results from our *in vitro* studies found that rolling cancer cells are more aggressive in nature with higher growth rate and invasion and form bigger and more colonies in soft agar and 3D sphere formation assays. In our *in vivo* study of orthotopic implantation mouse model, we found that sorted rolling cells are more potent in cases of tumor formation and distance metastasis. In summary, this flow based device provides a platform for isolating the most dangerous, aggressive form of cancer cells which can be used for isolating and characterizing human patients' peripheral blood and to form a process for customized cancer treatment.

15. SUBJECT TERMS

prostate cancer, metastasis, selectin, circulating, Micro-vascular

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Introduction

Prostate cancer is the second most common cause of male cancer death in the USA. The major cause of cancer patient mortality is metastasis. Recent studies found that epithelial origin cancer cells can undergo epithelial-to-mesenchymal transition (EMT), which plays an important role in metastasis, where the cancer cells gain the genes that can transform them to behave as 'leukocyte like' cells to be transported through the blood stream to their metastatic destinations (1). Like leukocytes, prostate cancer (PRCA) cells preferentially adhere and roll on bone marrow endothelial cells (BMECs) (2, 3) where abundant E-selectin is expressed (4), subsequently initiating a cascade of activation events that eventually leads to invasion, migration, and ultimately development of metastatic tumors (5-7). All of the observations support the notion that prostate cancer adhesion to the BMECs is a key step in the metastasis cascade. Selectin plays a very important role for capturing cancer cells from the blood stream and migrating them to the site of metastasis. (3). Activated prostate cancer cells become firmly adhered through integrin mediated interactions, and finally, the cells migrate across the endothelium under the direction of SDF-1 gradient (3). To study the mechanisms by which prostate cancer cells adhere to vessel endothelial cells, we applied a dynamic flow-based E-selectin coated microchannel system, cylindrical flow chamber. This selectin-mediated system mimics physiological events involved in normal cell trafficking such as recruitment of leukocytes during the inflammatory response and homing of hematopoietic stem cells to the bone marrow (8-10). We successfully established parameters for the dynamic flow-based E-selectin+SDF-1 coated microtubes, which can capture PRCA cells and allow us to study the circulating tumor cell behavior and its contribution to tumor metastasis. It was concluded that a tube coated with 40 µg/ml of E-selectin and 10µg/ml of SDF-1β with shear stress 1 dyne/cm^2 is the best possible combination to collect the smallest most potent PRCA cells from the tube based circulation model (preliminary data). We used this selectin and SDF-1β coated micro-renathane tubing system, which mimics pro-metastatic vascular endothelial cell surface, for the capturing of 'rolling/adhesion/attached' group of PRCA cells. We found that the 'rolling/adhesive/attached'group of cells possess more aggressive and stem cell type characteristics compared to the 'floating' cells in our *in vitro* assay. In summary, our experimental *in vitro* data confirmed that cell rolling is a critical step in controlling cancer metastasis, and this novel flow-based device provides a system to capture and enrich metastatic cancer cells from the circulation. Therefore, further evaluation is necessary not only to identify the pathways/genes that are responsible for cancer metastasis, but also to provide a pure population for preclinical drug screening. It opens up a process for customized cancer treatment which can have more potent action and also reduce the disadvantage of multiple drug side effects.

BODY

Task 1: Correlation of cancers' aggressiveness with their adhesion /rolling capacity in static and dynamic flow-based status (timeframe: months 1-12).

1a: Determination of cell rolling capacity in a dynamic state (timeframe: months 1-12).

1b: Determination of cell adhesion ability in a static condition (timeframe: months 1-8).

1c: Determination of cell metastatic behavior *in vitro* (timeframe: months 1-12).

Accomplishment: We completed task 1and reported this in our last report.

Task 2: Sorting prostate cancer cells into adhesion (attached) and non-adhesion (floating) subpopulations and comparison of these two populations' metastatic behaviors *in vitro* and *in vivo* (timeframe: months 5-24).

2a: Fractionation of prostate cancer (PRCA) cells based on rolling capacity (timeframe: months 5-20).

2b: Characterization of sorted cells metastatic behavior *in vitro* (timeframe: months 7-18).

2c: Confirmation of metastatic potential on those rolling PRCA cells *in vivo* by the orthotopic injection mice model (timeframe: months 13-24).

Accomplishment:

We completed task 2a and 2b and reported this in our last report.

Task 2c: Confirmation of metastatic potential on those rolling PRCA cells *in vivo* by the orthotopic injection mice model.

To discover the *in vivo* carcinogenic/metastatic aggressive behavior which we mentioned in Task 2C, we implanted freshly sorted 'attached/rolling (R)' and 'floating (F)' PC3 PRCA cells in the anterior prostate of total 10 nu/nu mice (Jackson Lab); 5 with 'attached/rolling (R)' and another 5 with 'floating (F)'$1X10^6$ cells/mouse with the approval of University of Rochester's UCAR protocol. Matrigel was used for dilution of the cells. We euthanized the mice after 90 days to assay the development of tumor. 5 out of 5 'rolling' cell implanted mice (RM) show prostatic tumor (Figure 1A), but strikingly, another 5 of the 'floating' cell implanted mice (FM) did not

show any visible tumor (figure 1B). All 'FM' show normal sized lymph nodes but 4 out of 5 'RM' show enlarged lymph nodes to the naked eye. 4 out of 5 RM also show metastatic tumor in their lungs and lymph nodes under microscope with H&E staining, where all 5 FM demonstrated intact basement membrane and glandular structure of the anterior prostate with normal lungs and lymph node structures (Figure 2A). Antibody to human pan cytokeratin (Abcam) shows positive signal for the RM tumor but not for the FM prostates (Figure 2B).

Figure 1

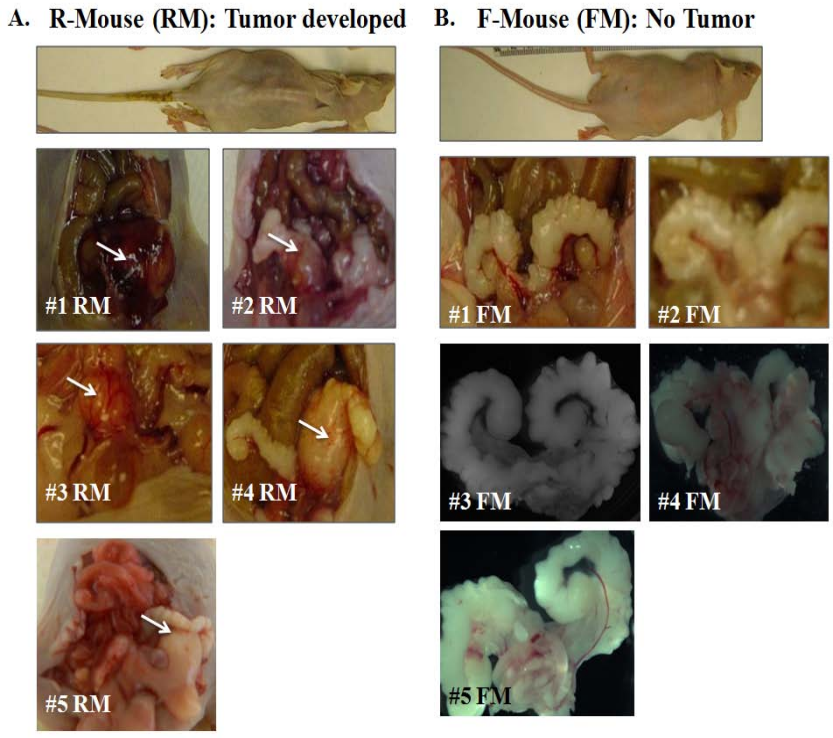

Figure 1. For orthotopic Xenograft model, freshly sorted PC3 PRCA cells were implanted in the anterior prostate of nu mice (10^6 cells for each type). 5 nu mice were implanted with 'rolling' PC3 cells and 5 nu mice with 'floating' PC3 cells. Mice were euthanized after 90 days 0f implantation. 5 out of 5 'rolling' cells implanted mice (RM) show big prostatic tumor (A). But all 5 of the 'floating' cell implanted mice (FM) did not show any tumor to the naked eye (B).

Figure 2

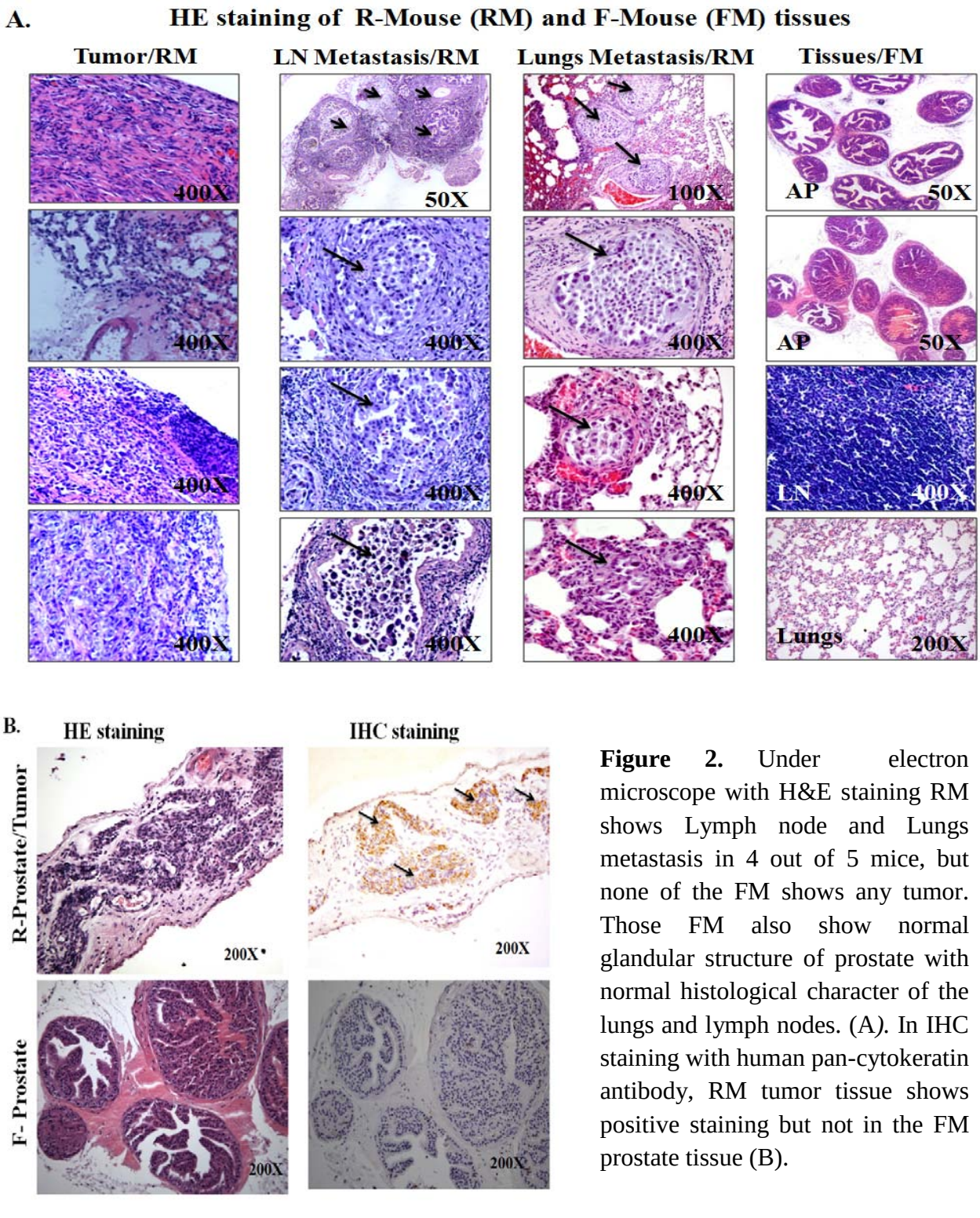

Figure 2. Under electron microscope with H&E staining RM shows Lymph node and Lungs metastasis in 4 out of 5 mice, but none of the FM shows any tumor. Those FM also show normal glandular structure of prostate with normal histological character of the lungs and lymph nodes. (A*).* In IHC staining with human pan-cytokeratin antibody, RM tumor tissue shows positive staining but not in the FM prostate tissue (B).

Task 3:

3a: Verify up-regulation of metastatic promoting genes in rolling PCa cells by the Scatter Plot analysis using the metastasis pathway SuperArray (timeframe: months 7-12).

3b: Using the genome wide gene array profiling to build the network that controls PCa cell adhesion and metastasis (timeframe: months 13-24).

3c: Determination of those metastatic related genes impact to cell rolling capacity and metastatic behavior *in vitro* (timeframe: months 15-30).

3d: Determination of those metastatic related genes' impact to cell rolling capacity and metastatic behavior *in vitro and in vivo* (timeframe: months 25-36).

Accomplishment:

Comparing the metastatic behavior in gene level between the rolling and floating PCa cell population. Human metastasis tumor focused gene array was performed to compare the gene profile alteration on these two sorted subpopulations. We sorted two circulating PRCA cell lines, PC3 and DU-145 cells, into 'rolling' and 'floating' subpopulation, and then RNA were extracted using Trizol (Invitrogen) and converted to cDNA. We then performed RT2 Profiler PCR human metastasis tumor focused gene array (SABioscience) to compare the gene profile alteration on these two sorted subpopulations to determine and compare up- and down-regulated metastatic genes among 'floating' and 'rolling/attached' cell population. 27 out of 84 metastatic genes were at least two fold up-regulated and 3 tumor suppressive genes were down-regulated in PC3 rolling/attached cell line (Figure 3A) and 14 out of 84 genes were also up-regulated in DU-145 cell line (Figure 3B). 7 common genes were identified between these 2 cell lines, PC3 and DU-145, using Venn Diagram model (Figure 3C) and qPCR was performed on some of those genes for further confirmation. 'Rolling' cells of both PC3 and DU-145 cell lines show higher expression. (Figure 3, D and E). Some prostate stem cell markers (CD 133, CK5 and CK8) and some endothelial mesenchymal transformation (EMT) markers were also upgraded in the rolling cell populations (Figure 4).

Figure 3

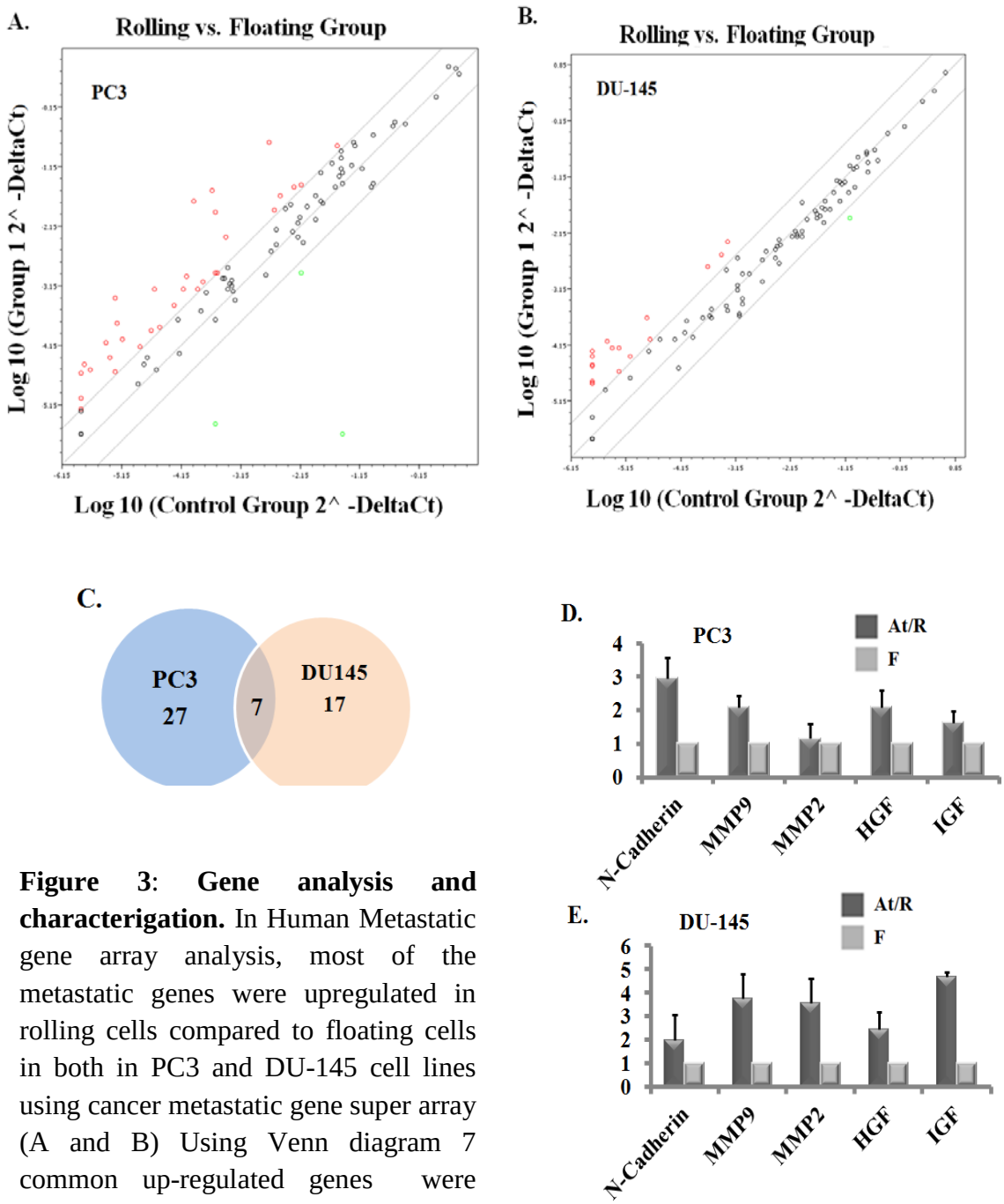

Figure 3: Gene analysis and characterigation. In Human Metastatic gene array analysis, most of the metastatic genes were upregulated in rolling cells compared to floating cells in both in PC3 and DU-145 cell lines using cancer metastatic gene super array (A and B) Using Venn diagram 7 common up-regulated genes were identified (C) and were confirmed by QPCR (D and F).

Figure 4

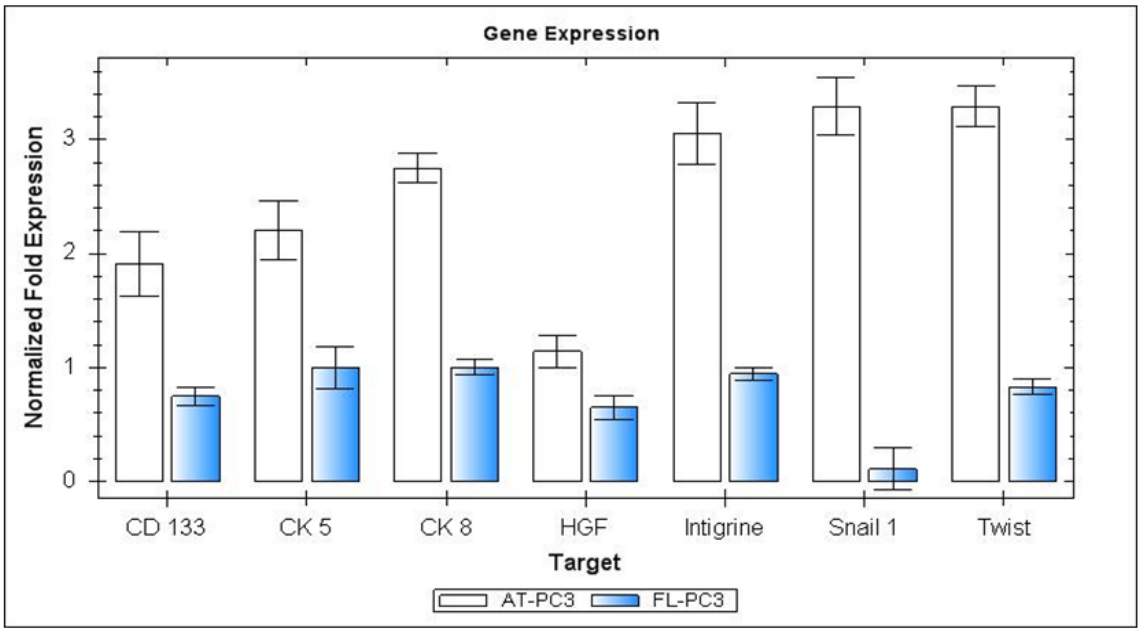

Figure 4. mRNA of some prostate specific stem cell (CD 133, CK5, CK 8) and EMT marker (snail 1 and twist) genes were also upgraded in the attached/rolling cell population.

Key Research Accomplishments

- Performing an orthotopic implantation xenograft model for the sorted ‘rolling’ and ‘floating’ prostate cancer cells.
- Performing human metastasis tumor focused gene array to compare the gene profile alteration on these two sorted subpopulations.
- Analyzing some prostate stem cell markers and endothelial mesenchymal transformation (EMT) markers on these two sorted subpopulations.

Reportable Outcomes

Participated in poster presentation at the University of Rochester Graduate Research Showcase 2013, University of Rochester, Thursday, April 18th, 2013.

Conclusion

Using this flow-based dynamic system we were able to capture a small portion of circulating PRCA cells that possess higher metastatic potential based on our *in vitro* and *in vivo* data. We will continue to work on discovering these metastatic related genes' impact on prostate cancer cell rolling capacity and metastatic behavior. This strategy provides a powerful way to capture and interrupt prostate cancer adhesion to the blood vessel during circulation, which might eventually slow down cancer progression. Furthermore, this dynamic functional fractionation system could be further extended as a general approach for sorting of diverse cancer cell populations based on differences in functional adhesion properties.

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