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Multicolor flow cytometric analysis of cancer-derived microvesicles reveals a unique subpopulation ratio in plasma from prostate cancer patients

*Jorge L Schettini, Mukesh Maheshwari, Krunal Desai, Brian Rhees and David B. Spetzler
Caris Life Sciences. 4610 South 44th Place, Phoenix, AZ 85040 (* jschettini@carisls.com)

Abstract

Circulating microvesicles (cMV) are membrane-bound cell-derived structures that can be isolated from many biofluids and culture media. Previous studies have shown that cMVs are released by several cell types including immunocytes, endothelial, embryonic, tumor cells and also platelets. cMVs in blood are a source of potential biomarkers of disease diagnosis and progression. The purpose of this study was to determine whether exposed biomarkers on the surface of cMVs from processed plasma could distinguish prostate cancer from atypia, high grade prostatic intraepithelial neoplasia (HGPIN), benign or prostate inflammation. Isolated cMVs from the blood of biopsy-positive cancer patients were stained with a panel of specific antibodies to compare phenotype, frequency and marker expression. Samples were collected prospectively prior to biopsy. The distribution of the cohort included 80 men with previously undiagnosed prostate cancer, 13 men with previously diagnosed prostate cancer (active surveillance), 6 atypia, 23 HGPIN, 28 inflammation, 49 benign and 25 normal samples. The cMVs from these patients were analysed by flow cytometry. Subpopulations of cMVs were determined based on the expression of multiple combinations of markers through proper gating. A systematic analysis of all potential biomarkers combinations showed a significant EpCAM positive subpopulation of cMVs in prostate cancer samples (current biopsy) and HGPIN/Atypia over other conditions. These results demonstrate that isolated cMVs from plasma can be used to determine relevant subpopulations in prostate cancer diagnosis.

Mat. & Methods

Microvesicles from plasma were obtained from patients and healthy donors by a blood draw according to Caris Life Sciences protocol. Samples: Plasma from 80 men with previously undiagnosed prostate cancer, 13 men with previously diagnosed prostate cancer (PCa-active surveillance), 6 atypia (atypical hyperplasia), 23 HGPIN (high-grade prostatic intraepithelial neoplasia), 28 inflammation, 49 benign and 25 normal samples (NA) were collected and processed for staining with fluorochrome-conjugated antibodies cocktail.

Antigen staining on microvesicles surface were done with 1ug of fluorochrome conjugated monoclonal antibodies cocktail: APC-EpCAM (Santa Cruz #), PE-PCSA (#), Atto425-Adam10 (#) and PE-Cy7-Adams10 (#) for 30 min on ice before acquisition.

BD FACSCanto™ II Flow cytometer was used to acquired all samples in this study. Data analysis was performed with Flow Jo v9.4 software (Tree Star, Inc)

Results

Analysis of microvesicles from plasma samples by a panel of specific monoclonal antibodies have shown in this cohort that biomarkers were expressed with a similar patter on several types of samples (PCa, Benign, normals, Inflammation, HGPIN, and Atypia) (Figure 1-2).

An extensive analysis of different combinations of these four biomarkers co-expressed on microvesicles we investigated. Results suggests that frequencies of co-expressed markers did not show a significant different between PCa samples and the rest of the cohort, with the exception of Atypia (Figure 3). Atypia samples have show an increased frequency of $PCSA^{+}Adam10^{+}$ double positive events on $EpCAM^{+}SSC^{HI}$-$EpCAM^{+}SSC^{LO}$ ratio (Figure 3 top right panel). Analysis of light side scattering on these microvesicles with EpCAM expression and positive for PCSA-Muc2-Adam10 suggests that cancer samples and HGPIN/Atypia have changed the ratio between these two subpopulations of microvesicles (Figure 4).

Conclusions

Based on previous experiments four biomarkers EpCAM, Muc2, Adam10 and PCSA were selected to study the phenotype of plasma microvesicles by flow cytometry. These markers were found to be expressed in similar fashion throughout this cohort. However, analysis of side scatter on triple positive expression of PCSA/Muc2/Adam10 has revealed two unique subpopulations based on SSC magnitude and EpCAM expression. These results suggested that different levels of microvesicles complexity could be found in cancer samples with potential prostate cancer diagnosis.

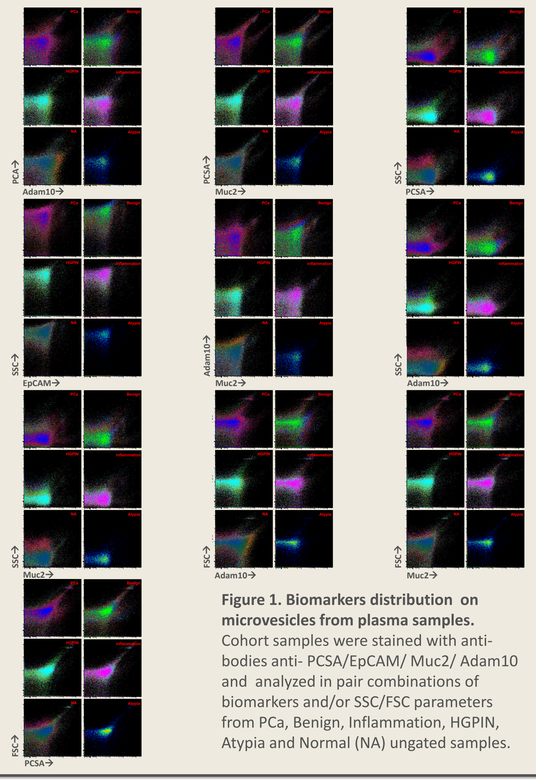

Figure 1. Biomarkers distribution on microvesicles from plasma samples. Cohort samples were stained with anti-bodies anti- PCSA/EpCAM/ Muc2/ Adam10 and analyzed in pair combinations of biomarkers and/or SSC/FSC parameters from PCa, Benign, Inflammation, HGPIN, Atypia and Normal (NA) ungated samples.

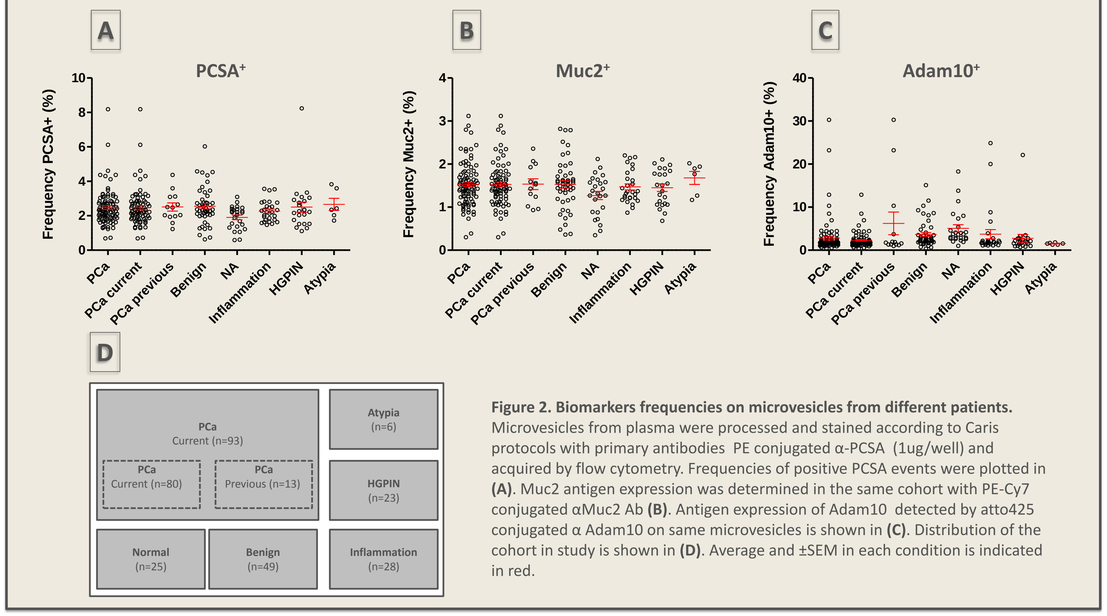

Figure 2. Biomarkers frequencies on microvesicles from different patients. Microvesicles from plasma were processed and stained according to Caris protocols with primary antibodies PE conjugated α-PCSA (1ug/well) and acquired by flow cytometry. Frequencies of positive PCSA events were plotted in **(A)**. Muc2 antigen expression was determined in the same cohort with PE-Cy7 conjugated αMuc2 Ab **(B)**. Antigen expression of Adam10 detected by atto425 conjugated α Adam10 on same microvesicles is shown in **(C)**. Distribution of the cohort in study is shown in **(D)**. Average and ±SEM in each condition is indicated in red.

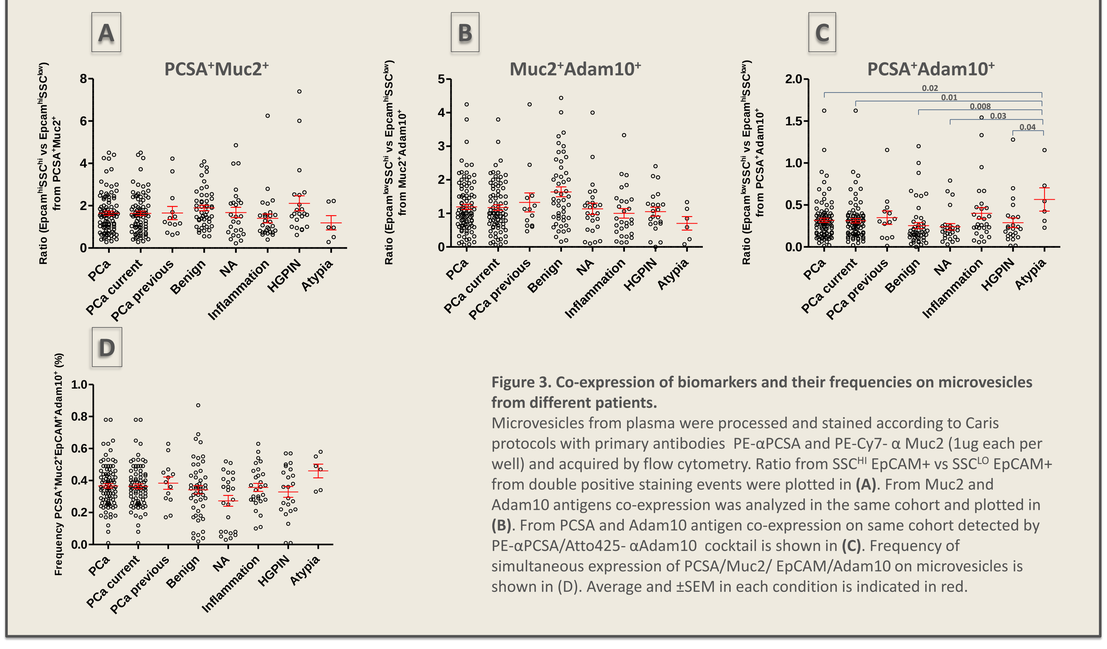

Figure 3. Co-expression of biomarkers and their frequencies on microvesicles from different patients. Microvesicles from plasma were processed and stained according to Caris protocols with primary antibodies PE-αPCSA and PE-Cy7- α Muc2 (1ug each per well) and acquired by flow cytometry. Ratio from SSC^{HI} EpCAM+ vs SSC^{LO} EpCAM+ from double positive staining events were plotted in **(A)**. From Muc2 and Adam10 antigens co-expression was analyzed in the same cohort and plotted in **(B)**. From PCSA and Adam10 antigen co-expression on same cohort detected by PE-αPCSA/Atto425- αAdam10 cocktail is shown in **(C)**. Frequency of simultaneous expression of PCSA/Muc2/ EpCAM/Adam10 on microvesicles is shown in (D). Average and ±SEM in each condition is indicated in red.

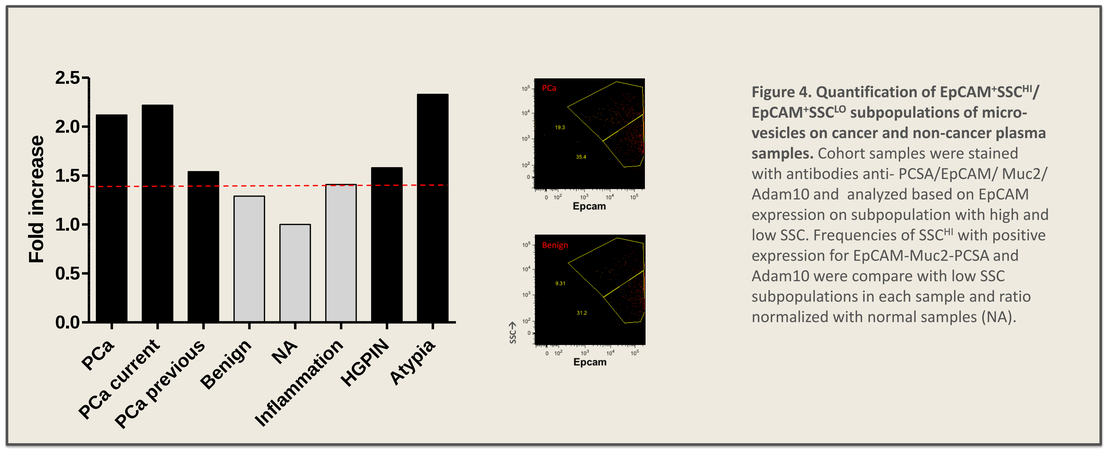

Figure 4. Quantification of $EpCAM^{+}SSC^{HI}$/ $EpCAM^{+}SSC^{LO}$ subpopulations of micro-vesicles on cancer and non-cancer plasma samples. Cohort samples were stained with antibodies anti- PCSA/EpCAM/ Muc2/ Adam10 and analyzed based on EpCAM expression on subpopulation with high and low SSC. Frequencies of SSC^{HI} with positive expression for EpCAM-Muc2-PCSA and Adam10 were compare with low SSC subpopulations in each sample and ratio normalized with normal samples (NA).