

Subpopulations of Circulating Microvesicles Have Different MicroRNA Profiles

Ta Deng, David B. Spetzler*, Daniel Holterman, Traci Pawlowski, Janet E. Duncan, Shannon E. Smith, Katrina Radebach, Christine D. Kuslich.

Caris Life Sciences. 4610 South 44th Place, Phoenix, AZ 85040 (www.Carislifesciences.com)

Abstract

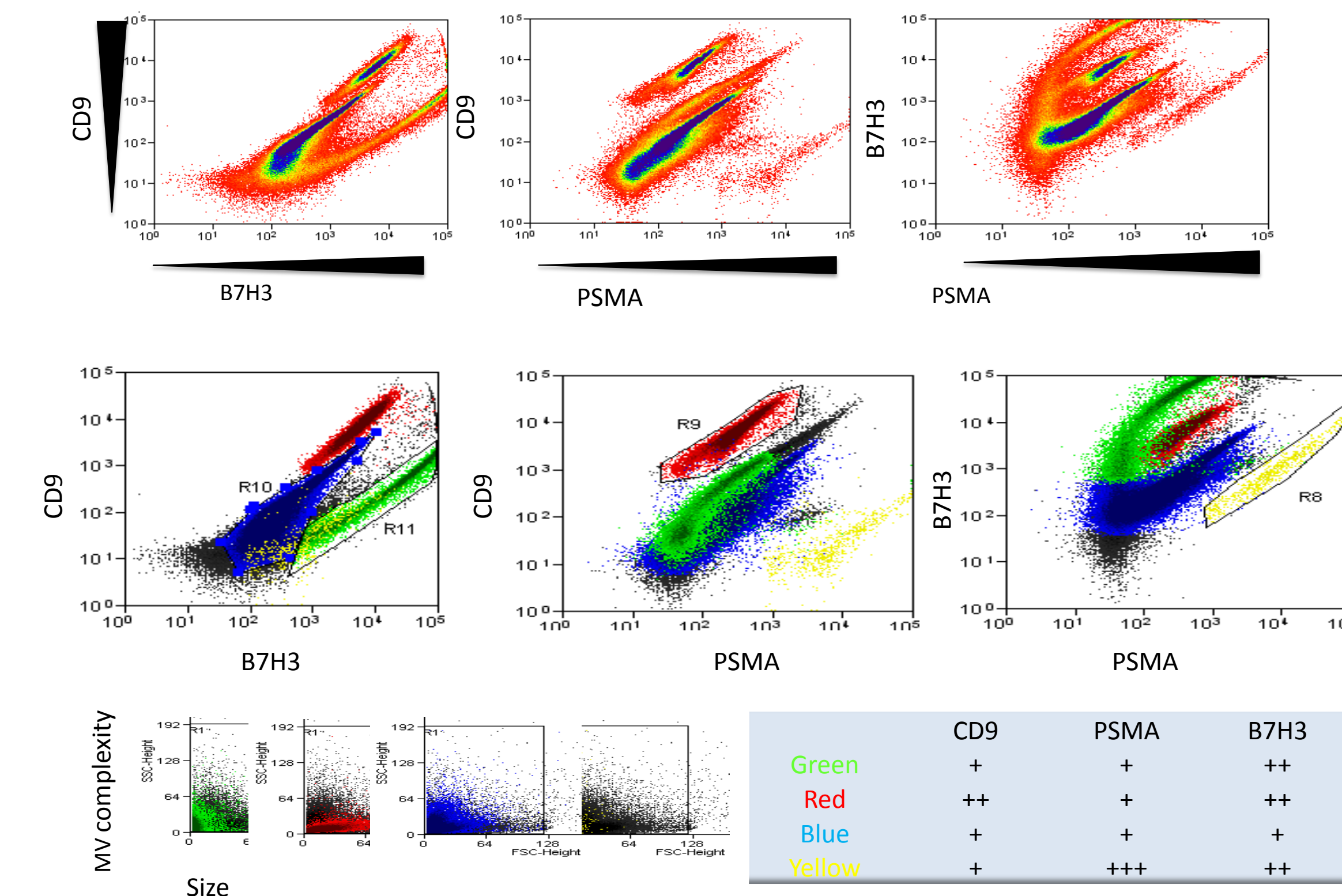
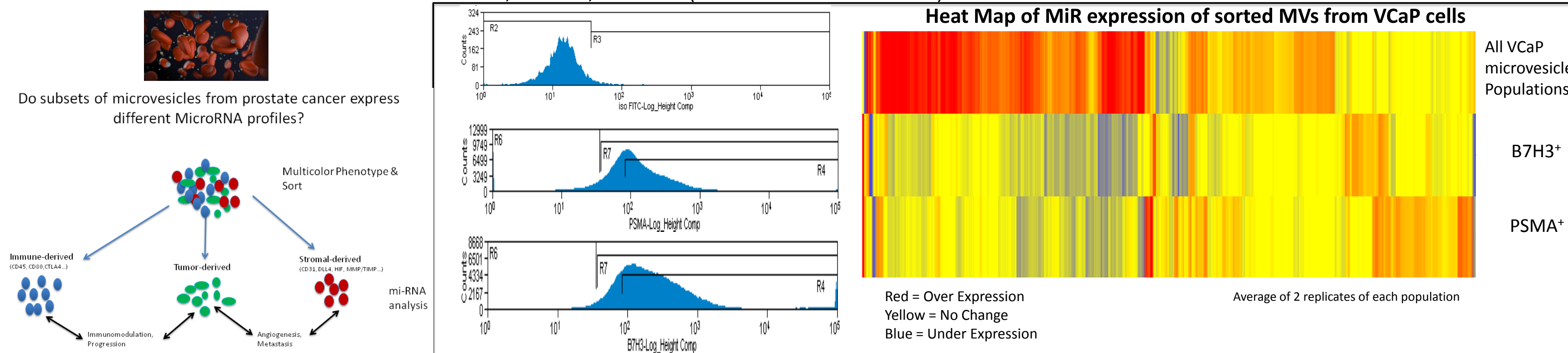
The purpose of this study was to determine if microvesicle (MV) subpopulations as defined by surface protein composition also exhibit distinct microRNA (miR) expression patterns. Microvesicles isolated from a prostate cancer cell line (VCaP) were sorted based on their surface protein composition and were evaluated for differential expression of miRs. Phycoerythrin-labeled antibodies targeting EpCAM, CD63, or B7-H3 were used to sort the subpopulations of MVs by fluorescence-activated cell sorting. The sorted subpopulations of MVs were subsequently profiled by miR expression. The miR profiles for the EpCam-, CD63-, and B7-H3-positive subpopulations were compared with the profile of the total VCaP MV population. Differential miR expression patterns were observed across the subpopulations, and all expression patterns were distinct from that observed in the total population. Patterns of both over- and under-expression were seen. Not only can subpopulations of MVs be distinguished and separated based on surface protein markers, but they can also be differentiated based on their RNA content, in this case miRs. The ability to isolate tissue-specific cMV populations from patient plasma based on surface protein composition and then analyze them based on both surface protein composition and genetic content has considerable diagnostic, prognostic, and theranostic implications.

Methods

Microvesicles were isolated from tissue culture supernatant from confluent VCaP cells. VCaP is an androgen sensitive human prostate cancer cell line established in 1977 from prostate cancer tissue harvested from a metastatic lesion to a lumbar vertebral body of a patient with hormone refractory prostate cancer. The microvesicles were isolated using centrifugation of 400xg at 4°C for 10 minutes to remove cells followed by centrifugation of 2,000xg at 4°C for 20 minutes to remove cellular debris. Then the supernatant was concentrated using a centrifugal filter unit (Millipore Centricon Plus) with 1000xg at room temperature for 30 minutes to enrich for microvesicles. The enrichment was followed by purification of the MVs using a 30% sucrose solution centrifuged 100,000xg for 75 minutes at 4°C. MVs were stored -70°C until ready for phenotyping.

Staining of the VCaP MVs were performed by mixing aliquots of MVs with combinations of three fluorochrome-conjugated Antibodies (Abs) as indicated and analyzed with a Beckman-Coulter Mo-Flow XDL cytometer. The histograms were backgated using Summit flow cytometry software to show relative co-expression of the various subpopulations of microvesicles isolated from VCaP cells. Additionally, the MVs were sorted by the flow cytometer according to B7H3 and PSMA expression.

Then miR expression of unsorted B7H3⁺ and PSMA⁺ sorted microvesicles were compared using Exiqon RT-PCR panel and a heat map was generated using GeneSpring GX Software.



Results

The results of these studies demonstrate that MVs isolated from human prostate cancer can be phenotyped using a flow cytometer. Importantly, MVs from a homogenous cell line do not produce homogenous MVs. Rather, using 3-color analysis, four distinct subpopulations were detected based on expression of CD9, PSMA and B7H3. Using post-acquisition color-gating software it was determined that the four groups were consistent in their relative co-expression of these markers. For example, group 1 (colored green) was shown to be weakly positive for CD9 and PSMA and moderately positive for B7H3. Group 2 (colored red) was shown to be moderately positive for CD9 and B7H3 and weakly positive for PSMA. Group 3 (colored blue) was weakly positive for all three markers and group 4 (yellow) was weakly positive for CD9, strongly positive for PSMA and moderately positive for B7H3. This raised the question of whether these different subpopulations of prostate cancer-derived MVs contain differing populations of miRs.

To address this question the MVs were sorted by the flow cytometer according to PSMA or B7H3 expression. Approximately 10⁶ antigen positive MVs were sorted for miR analysis using the Exiqon miR RT-PCR panel. Additionally, 10⁶ unsorted VCaP microvesicles were evaluated for comparison. The results, expressed as a heat map for relative expression levels compared with a standard, demonstrate ~70% of the evaluated miRs were lost in both sorted populations, ~5% are enriched in B7H3⁺, and ~10% are enriched in PSMA⁺ MVs.

Conclusions

These studies demonstrate that microvesicles produced by the human prostate cell line VCaP are heterogeneous with regard to relative expression of the surface markers CD9, PSMA and B7H3. Additionally, when these microparticles were sorted by surface protein expression levels distinct subpopulations of miRs were identified.