Multiplexed Bead-based Extracellular Vesicle Profiling of Aqueous Humor from Uveal Melanoma Eyes

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Introduction

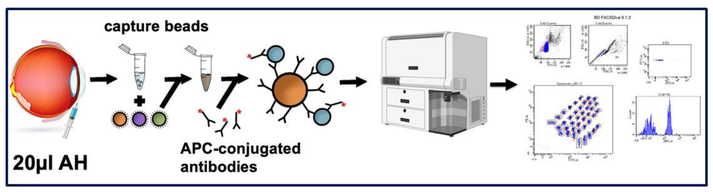
Extracellular vesicles (EVs) are small vesicles crucial for cellular communication and have potential as cancer biomarkers. However, a standardized analysis method using multiplexed bead-based flow cytometry for EVs in aqueous humor (AH) is yet to be established.

This study aims to utilize intraocular EVs in AH as a liquid biopsy platform for uveal melanoma (UM) patients. The stability and accessibility of AH make it an attractive option to monitor UM progression and metastasis over time.

MPA$_{PASS}$ is an innovative framework developed to analyze EV profiles using multiplexed bead-based assays. Our objective is to evaluate MPA$_{PASS}$ as a universally accepted tool for analyzing EV data in AH samples from UM patients.

Methods

- Our dataset included 29 AH samples collected from:
  - ❑ 24 uveal melanoma diagnostic samples
  - ❑ 3 retinoblastoma samples
  - ❑ 2 glaucoma samples
- 20 µL of each AH sample was subject to multiplexed bead-based EV profiling (MACSPlex).
- This data was analyzed on MPA$_{PASS}$ after fold change normalization (log-scaled).



The diagram above is an illustrative workflow for multiplexed bead-based flow cytometry to analyze surface markers on EVs.

References

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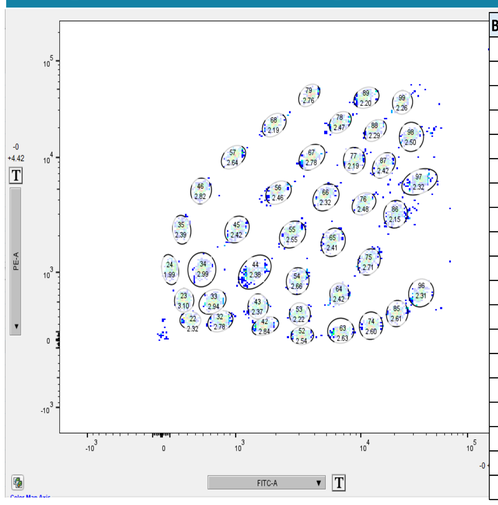
• Im DH, Peng CC, Xu L, Kim ME, Ostrow D, Yellapantula V, Bootwalla M, Biegel JA, Gai X, Prabakar RK, Kuhn P, Hicks J, Berry JL. Potential of Aqueous Humor as a Liquid Biopsy for Uveal Melanoma. Int J Mol Sci. 2022 Jun 2;23(11):6226. PMID: 35682905

Acknowledgements

• Knight Templar Eye Foundation
• CHLA TSRI RCDA
• Norris Comprehensive Cancer Center, USC, LA
• CHLA/KECK SORF Program
• TriDelta
• Concern Foundation

Figure 1: Gating Flow Cytometry Data



Bead Number	Bead Antibody	Bead Number	Bead Antibody
22	CD3	64	CD11C
23	CD4	65	CD81
24	CD19	66	AN2
32	CD8a	67	CD146
33	MHC Class II	68	CD41
34	CD49b	74	CD61
35	CD105	75	CD24
42	CD2	76	CD86
43	CD11b	77	CD44
44	CD25	78	CD326
45	CD49e	79	Prominin-1
46	CD140a	85	CD29
52	CD66a	86	CD69
53	CD9	87	CD142
54	CD205	88	CD45
55	MHCI	89	CD31
56	CD63	96	REA Control
57	CD40	97	CD20
63	CD62P	98	CD115
		99	EphA2

Figure 1 shows an example of data generation after gating flow cytometry data (UM 43). Values represent fluorescence intensity in the *FITC-A* and *PE-A* detectors. This fluorescent signature allows us to identify EVs that display specific markers, corresponding to our beads.

After excluding cell debris for each *SSC vs FSC* scatter plot, we performed similar circular gating for all 39 bead populations on *FITC-A* and *PE-A* channels in each bivariate dot plot. The respective bead legend is based off the *Miltenyi biotec MACSPlex Exosome* protocol.

Figure 2: Clustering of UM, GLC, and RR

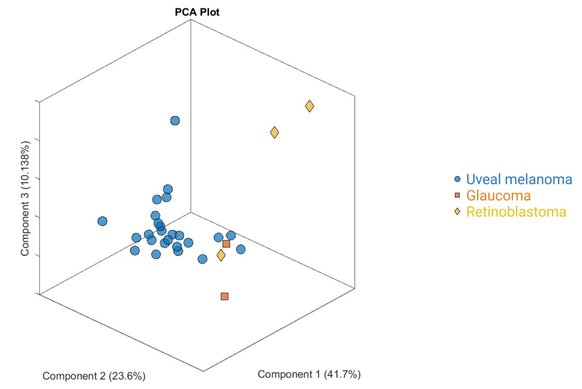
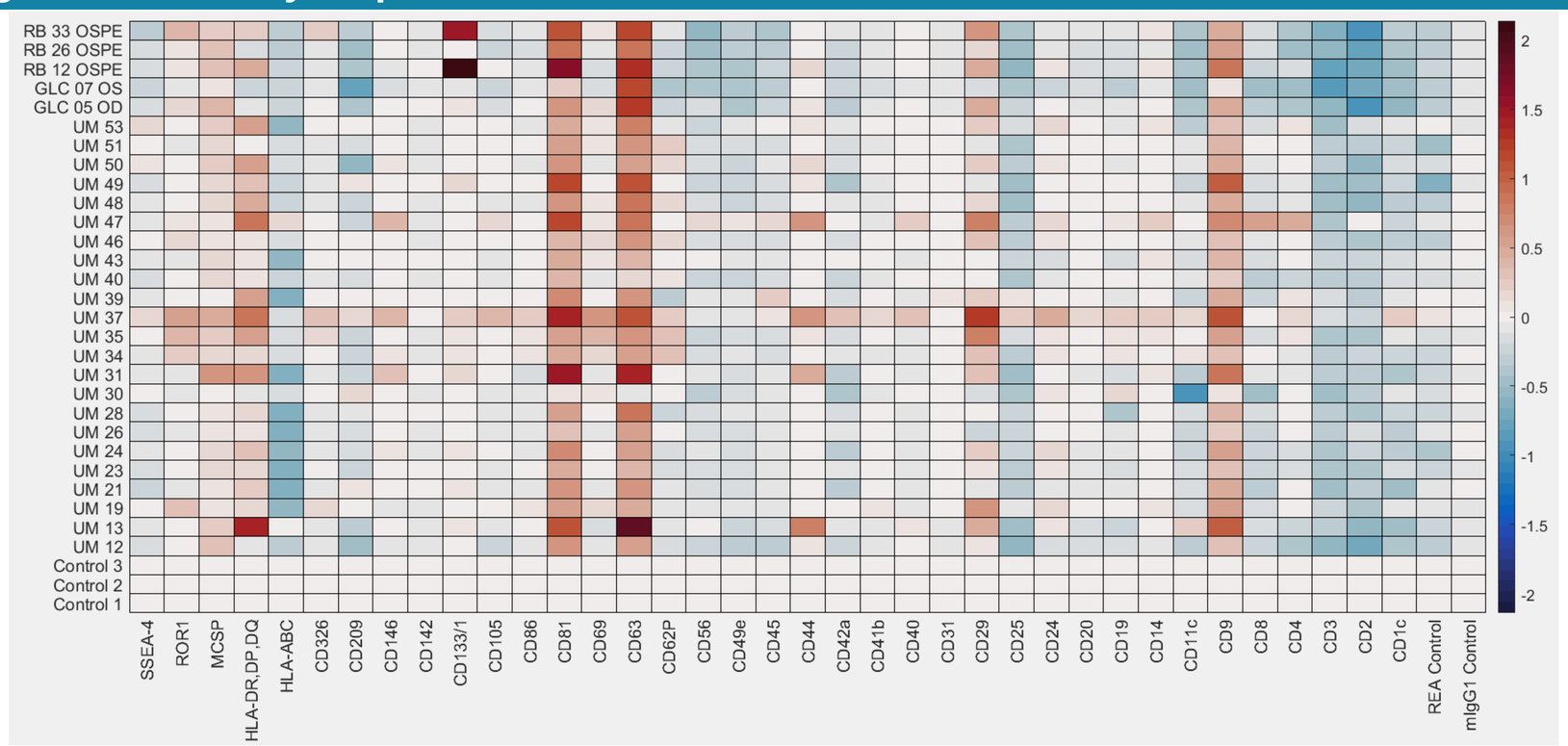


Figure 2 is a 3D PCA plot generated by MPA$_{PASS}$ which shows AH samples from different diseases are unsupervised clustered.

Conclusion and Future Directions

- Robust magnetic bead-based EV profiling enables detailed statistical analysis and clinical marker associations
- Disease-specific EV expression observed (UM: HLA-DR/DP/DQ, CD29, MCSP; RB: CD133/1)
- Further statistical analysis is warranted to fully explore the implications of the observed disease-specific EV expression patterns.
- Future research plans include comparing the coexpression patterns of tetraspanins at the single vesicle resolution with previous findings, to gain deeper insights into EV characteristics.

Figure 3: Intensity Map of EVs



In Figure 3, the authentic surface markers of small extracellular vesicles (EVs), namely tetraspanins - CD81, CD63, and CD9, are expressed with a significantly high enrichment ratio as anticipated. The three most enriched surface markers specific to UM identified in this analysis are: **HLA-DR/DP/DQ,** a major histocompatibility complex (MHC) class II molecule responsible for immune recognition of UM; **CD29** (Integrin β1), which may be associated with UM tumor progression; and **MCSP** (Melanoma-associated Chondroitin Sulfate Proteoglycan), a marker used for capturing circulating tumor cells of UM. **CD133/1** represents a unique marker of cellular differentiation for RB patients.