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

BACKGROUND: 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. The stability and accessibility of AH make it an attractive option to monitor UM progression and metastasis over time. Because it is difficult to biopsy small tumors in the macula without retinal complications, liquid biopsy of AH may be favored to mitigate these risks. This study aims to utilize intraocular EVs in AH as a liquid biopsy platform for uveal melanoma UM patients.

OBJECTIVE: 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 potential, universally accepted tool for analyzing EV data in AH samples from UM patients.

METHODS: Our samples were collected at the Melanoma Clinic in the Roski Eye Institute at Keck. All 24 participants provided informed consent, and their AH was sampled with corneal paracentesis and fine needle aspiration biopsy. 20μL of AH was subject to multiplexed bead-based EV profiling (MACSPlex),before our flow cytometry data was gated appropriately in FlowJo. This processed data was then analyzed in MPA_{PASS}.

RESULTS: We observed expression of the tetraspanins (CD9, CD63, and CD81), and disease-specific EV expression among UM patients (HLA-DR/DP/DQ, CD29, and MCSP). Our heatmap yielded additional information on CD133/1 expression as a promising marker for retinoblastoma patients.

CONCLUSIONS: 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.