Correlation of Pathogenic Somatic Mutation Presence in Tumor Tissue and Plasma-Derived Extracellular Vesicles

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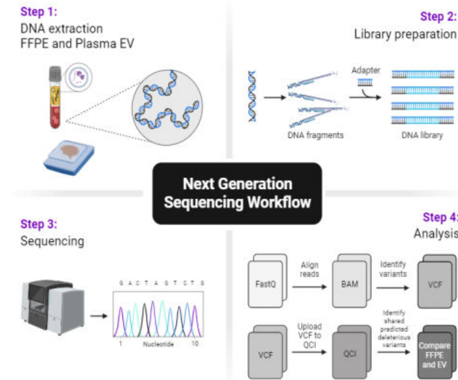
Introduction

Current liquid biopsy strategies have demonstrated value in clinical decision-making by enabling earlier cancer detection, diagnosis, and treatment monitoring. Cell-free circulating tumor DNA is elevated in the peripheral blood of cancer patients and liquid biopsy approaches most frequently target this tumor-derived analyte. Extracellular vesicles (EVs) are lipid membrane encapsulated particles that carry heterogeneous protein, nucleic acid, and metabolite cargos. Plasma EVs derived from tumor cells carry tumor-derived DNA. However, there is minimal data demonstrating the clinical utility of EV DNA derived from patient plasma. To demonstrate the value of EV DNA, we have compared tumor tissue somatic variants with pair-matched plasma EV DNA somatic variant calling data across three different cancers. We also compared variants identified in cell-free DNA (cfDNA) with EV DNA from the same patient plasma in select samples.

Methods

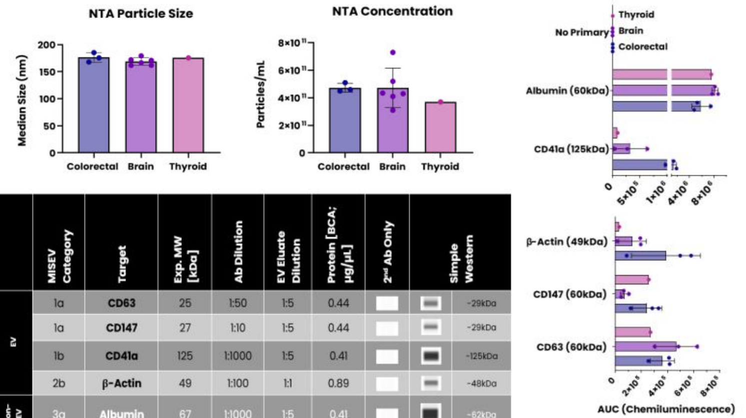


Formalin-fixed, paraffin-embedded (FFPE) tissue and matched plasma samples from brain, colorectal, and thyroid cancer patients were obtained. DNA was extracted from FFPE tissue. Plasma EVs were isolated and characterized according to MISEV guidelines. cfDNA and EV DNA libraries were constructed utilizing the Watchmaker DNA Library Prep Kit (ERAT) and tumor tissue DNA libraries were constructed utilizing the Watchmaker DNA Library Prep Kit with Fragmentation and sequenced on the Element Biosciences AVITI using 2x150. The somatic cancer workflow in QCI Interpret Translational was utilized to derive clinical significance and pathogenicity information.

Diagnosis	Count	Tumor Stage/Grade	Avg Tumor %	Avg Necrosis %	Average Age	Sex (M/F)
Brain Cancer	6	G1-G4	78.33	2.83	49.5	3/3
Colorectal Cancer	3	IIIB/G1-G3	63.3	3.33	64.66	2/1
Thyroid Cancer	1	IVA	90	0	74	0/1

Extracellular Vesicle Characterization

Nanoparticle tracking analysis (NTA) was used to verify EV particle size and concentration. Molecular characterization of EV protein content was performed using capillary electrophoresis Western blotting.



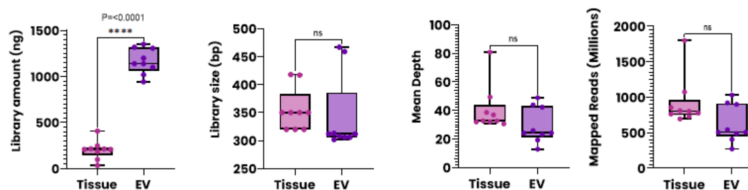
	MISEV Category	Target	Exp. MW [kDa]	Ab Dilution	EV Eluate Dilution	Protein [BCA; μg/μL]	2nd Ab Only	Simple Western
EV	1a	CD63	25	1:50	1:5	0.44		~29kDa
	1a	CD147	27	1:10	1:5	0.44		~29kDa
	1b	CD41a	125	1:1000	1:5	0.41		~125kDa
	2b	β-Actin	49	1:100	1:1	0.89		~48kDa
Non-EV	3a	Albumin	67	1:1000	1:5	0.41		~62kDa

Library Prep & Sequencing

Total Samples	Average Read Length	Average Coverage	Average Reads (Millions)
10 FFPE	130.54	33.07	684.86
10 EV	146.89	29.35	620.00

Quality Parameters

Quality parameters of libraries were assessed using Qubit fluorimeter and Bioanalyzer 2100. Sequencing quality metrics were recorded for the mean depth of coverage and the number of mapped reads.



Clinically Pathogenic Variants found in Samples

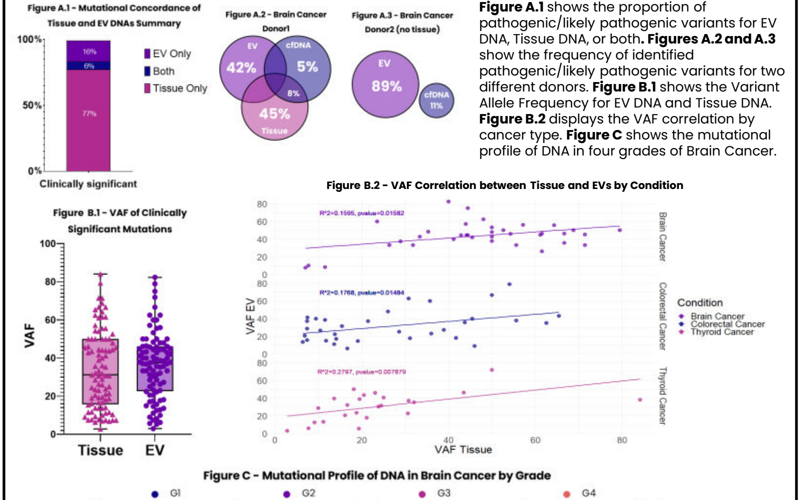


Figure A.1 shows the proportion of pathogenic/likely pathogenic variants for EV DNA, Tissue DNA, or both. **Figures A.2 and A.3** show the frequency of identified pathogenic/likely pathogenic variants for two different donors. **Figure B.1** shows the Variant Allele Frequency for EV DNA and Tissue DNA. **Figure B.2** displays the VAF correlation by cancer type. **Figure C** shows the mutational profile of DNA in four grades of Brain Cancer.

Conclusions

This study demonstrates that EV DNA can serve as a minimally invasive tool to gather clinically significant variant information that is traditionally obtained via tissue biopsy. Additionally, EV DNA yields less benign and likely benign variants but more variants of uncertain significance, which warrant further investigation. The preliminary data suggests that both EVs and FFPE tissue contain minimal overlap in pathogenic or likely pathogenic variants, but both contain clinically relevant unique variant information. cfDNA from plasma was analyzed and yielded fewer clinically significant variants than EV-isolated DNA, indicating that EVs can be used to identify clinically significant variants that are not present in cfDNA.

References/Acknowledgements

Thery et al. (2018). Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and an update of the MISEV2014 guidelines.
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All other graphs used GraphPad Prism for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.cmo
The workflow graphic figures were created with BioRender.

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