BioFluidica
The Future of Liquid Biopsy

APPLICATION NOTE

Fully automated isolation of Extracellular Vesicles Using LiquidScan® and Instrumentation for Molecular Subtyping of Breast Cancer

INTRODUCTION

Extracellular vesicles (EVs) are relatively high abundance markers for liquid biopsy in cancer disease management, detectable even in early-stage disease[1]. EV-associated RNA can reflect changes in RNA expression of the cells that produced them[2–4]. However, most methods for EV enrichment target all EVs, regardless of cellular source. This means that low-abundance mRNA transcripts, whose expression changes could serve as a disease indicator, are diluted among the total RNA carried by non-diseased EVs. To overcome this problem, BioFluidica created microfluidic chips surfaced with monoclonal antibodies (mAbs) that target disease-specific EV surface proteins[5–6]. In this application note, we applied a dual affinity selection strategy using anti-EpCAM and anti-FAPα (Fibroblast Activation Protein alpha) mAbs to enrich two orthogonal tumor-derived EV subpopulations sourced from epithelial cells and cells with a mesenchymal phenotype. The purified EVs were then used for exo-mRNA transcript analysis via RT-ddPCR to determine exo-mRNA abundance associated with a particular molecular subtype of breast cancer, which was determined by PAM50 assay run on the paired Formalin-fixed paraffin-embedded (FFPE) tumor tissue[7].

MATERIAL AND METHODS

Automated isolation of circulating liquid biopsy biomarkers: BioFluidica LiquidScan is an automated instrument isolating three different liquid biopsy biomarkers (cells, EVs, and cfDNA) using microfluidic chips that can be tuned for individual isolation tasks. For the high-affinity EV isolation, the **EV-MAP chip** (7 beds populated with 1.5M pillars) was used with immobilized mAbs.

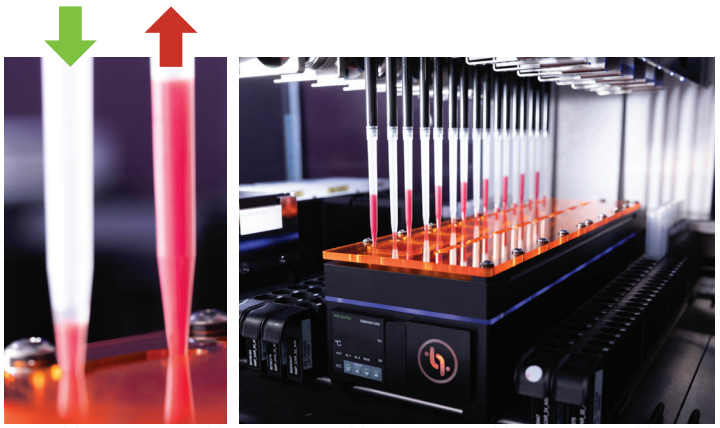
Affinity capture through mAbs: mAbs used in this work included mouse anti-human EpCAM mAb (R&D Systems, clone# 158210), mouse anti-human FAPα mAb (R&D Systems, clone# 427819), and mouse anti-human CD81 mAb (R&D Systems, clone# 454720) for the isolation of non-targeted EVs – pan EVs containing the mixture of all EVs as controls.

Patient plasma samples: Plasma from two male and two female healthy donors were obtained from Bio reclamation IVT. 6 deidentified cancer-free healthy participants and 11 breast cancer patients (IRB approved protocols) plasma samples were secured from the University of Kansas Medical Center (KUMC) Biospecimen Repository Core Facility.

EV Analysis: Multiple methods were used to analyze the particles through Nanoparticle tracking analysis (NTA), Transmission electron microscopy, and RNA quantitation. The molecular analysis was performed using ddPCR assays (BioRad) and Nanostring PAM50 assay on the nCounter platform. The molecular subtypes based on tumor tissue were determined using the published algorithm (developed to support the clinical test known as PAM50 or Prosigna®).

RESULTS

- The EV-MAP provided recovery >80% with a specificity of 99 ±1% based on exosomal mRNA (exo-mRNA) and RT-ddPCR results.
- On average, 0.5 mL of plasma from breast cancer patients yielded ~2.25 ng of total RNA for both EV^{EpCAM} and $EV^{FAPα}$. In contrast, in the case of cancer-free individuals, it yielded 0.8 ng and 1.25 ng of total RNA from EV^{EpCAM} and $EV^{FAPα}$, respectively.
- EVE^{pCAM} and $EV^{FAPα}$ exo-mRNA profiling using subsets of the PAM50 genes, and a novel algorithm generated 100% concordance with the tumor tissue.
- When using EV^{CD81} isolated EVs, the breast cancer subtype identification accuracy was 75%, irrespective of the gene subset(s) used.



A Pumping of the sample and reagents through microfluidic architecture is achieved by using two pipetting channels simultaneously, one in push (dispense) and one in pull (aspirate) mode.

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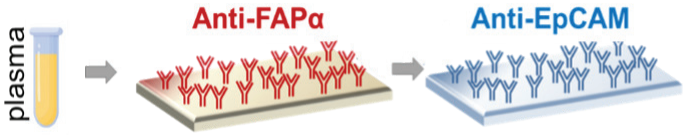
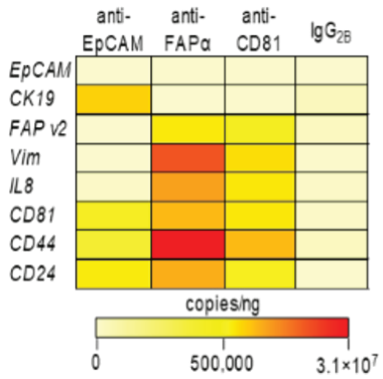
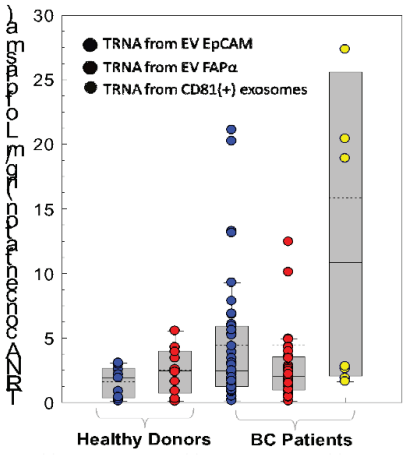


B Affinity isolation of EVs performed in a serial fashion.



C High expression of *CK19* and low expression of the mesenchymal-associated *FAPv2* and *VIM* in the anti-EpCAM isolate, but low expression of *CK19* and high expression of *FAPv2* and *VIM* for the anti-FAPα isolate. *CD44* (high)/ *CD24* (low) occurred in the anti-FAPα fraction, correlating well with the mesenchymal phenotype.



D – Box plots present the total RNA concentration extracted from isolated EV^{EpCAM} and $EV^{FAPα}$ from healthy donors and breast cancer patients' plasma.

CONCLUSIONS

- BioFluidica LiquidScan liquid handling robot enables high-throughput and fully automated plasma sample processing for EV isolation.
- The EVs isolated with BioFluidica EV-MAP microfluidic chips exhibit high capture efficiency, purity, and specificity.
- The dual-selection EV-MAP procedure and liquid biopsy samples were used to demonstrate the clinical feasibility of using EV for molecular subtyping breast cancers.
- Tissue-specific EV capture better represented the original gene signature than pan-EV capture.

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