Linking Extracellular Vesicle Markers to Cancer-Specific Biomarkers: A Correlative Study

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Introduction

- Extracellular vesicles (EVs) are rich sources of disease-related biomarkers (proteins, nucleic acids, metabolites). EVs offer promise for early detection, risk stratification, and screening.
- Current understanding of EV markers is guided by ISEV, but their link to cancer-specific biomarkers is underexplored. Most cancer biomarker studies have emphasized free-floating proteins and nucleic acids, not EVs proteins.
- Our pilot study investigates the relationship between EV markers and cancer-related proteins across multiple cancer cell-line types.
- A description of the AC Electrokinetics (ACE) platform has been previously published (Hinestrosa et al. *Front. Bioeng. Biotehnol.* **2020**, *8*, 581157).

Materials and Methods

EVs were isolated via ultracentrifugation from a selection of cancer cell lines: **H1975** (non-small cell lung adenocarcinoma), **A549** (lung adenocarcinoma), **AsPC-1** (pancreatic adenocarcinoma), and **SK-OV-3** (ovarian adenocarcinoma). Multiplex ELISA was used to measure EV markers (CD63, CD9, CD81) and 52 cancer-associated markers. We analyzed the EV markers and cancer biomarkers, in relation to EV particle concentration. Multiple concentration levels, spanning two or three orders of magnitude, were tested for each cell line. Subsequently, the EVs were spiked into PBS buffer, and CA19-9 biomarker level was assessed using an AC Electrokinetics (ACE) microelectrode array platform capable of probing the EVs via tagging with fluorescent antibodies.

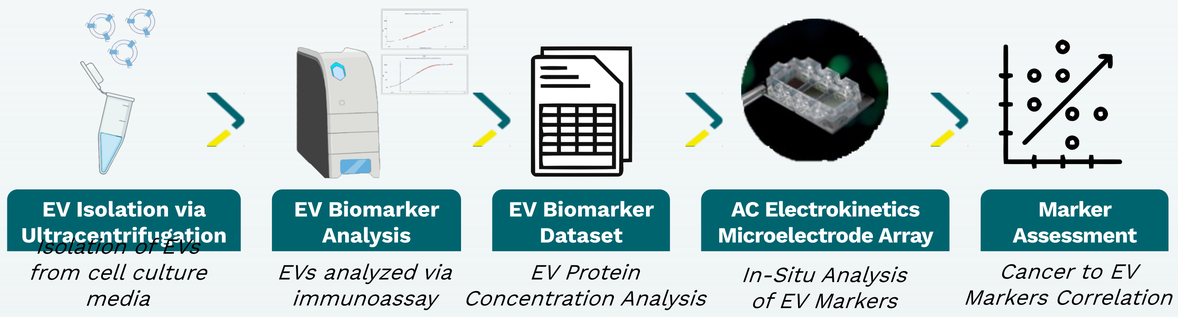

Figure 1. Workflow Overview. Extracellular Vesicles (EVs) were isolated from cell culture media using ultracentrifugation and then analyzed via immunoassays for a selection of 52 cancer biomarkers and the EV markers CD9, CD63 and CD81 on a commercial platform. The EV proteomic results were used to correlation analysis between the EV and Cancer biomarkers and analyzed for CA19-9 on the ACE platform.

Table 1. Cancer Cell Line Information

Cell Line Name	Cancer Type	ATCC ID	Particle Concentration (particles/mL)	Average Particle Size (nm)
A549	Lung Adenocarcinoma	CCL - 185™	4.4×10^{10}	135.8
AsPC-1	Pancreatic Adenocarcinoma	CRL - 1682™	1.1×10^{11}	122.9
H1975	Non-Small Cell Lung Adenocarcinoma	CRL - 5908™	2.7×10^{11}	158.6
SK-OV-3	Ovarian Adenocarcinoma	HTB - 77™	2.1×10^{11}	128.6

Results

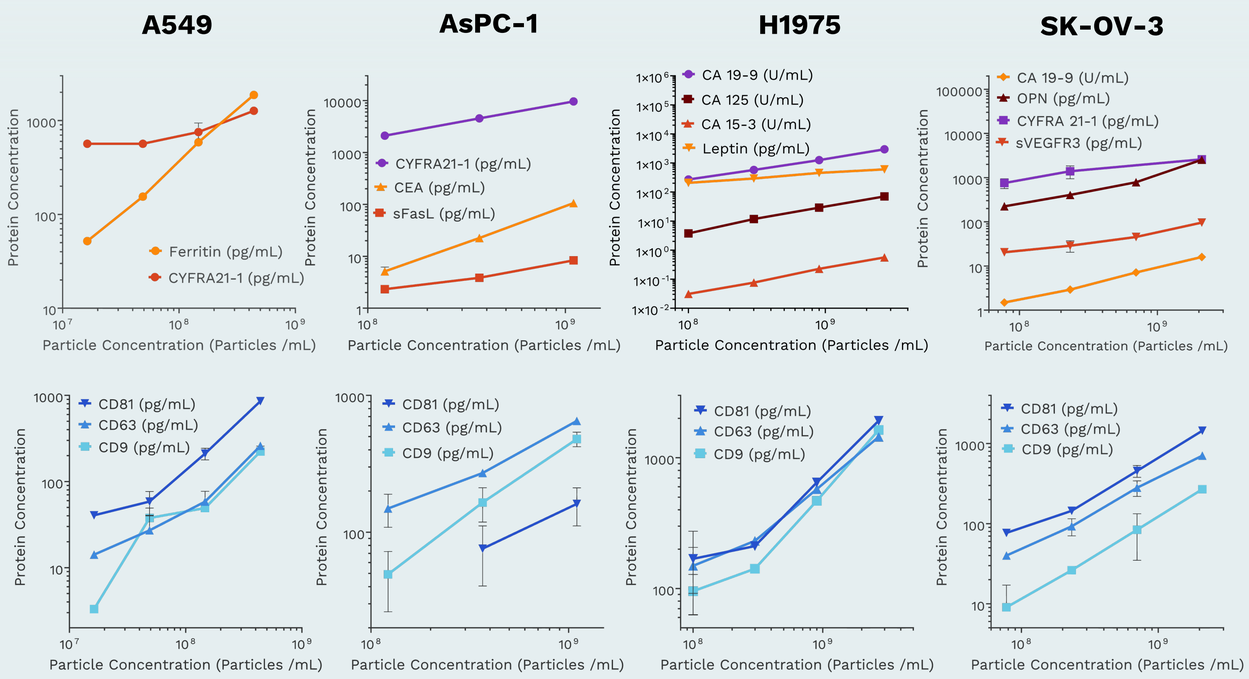

Figure 2. Biomarker readings for each cell line EV against particle concentration. (TOP) Cancer markers concentration for a selection of markers deemed a positive for expression on each cell line. (BOTTOM) Readings of CD9, CD63 and CD81.

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Results

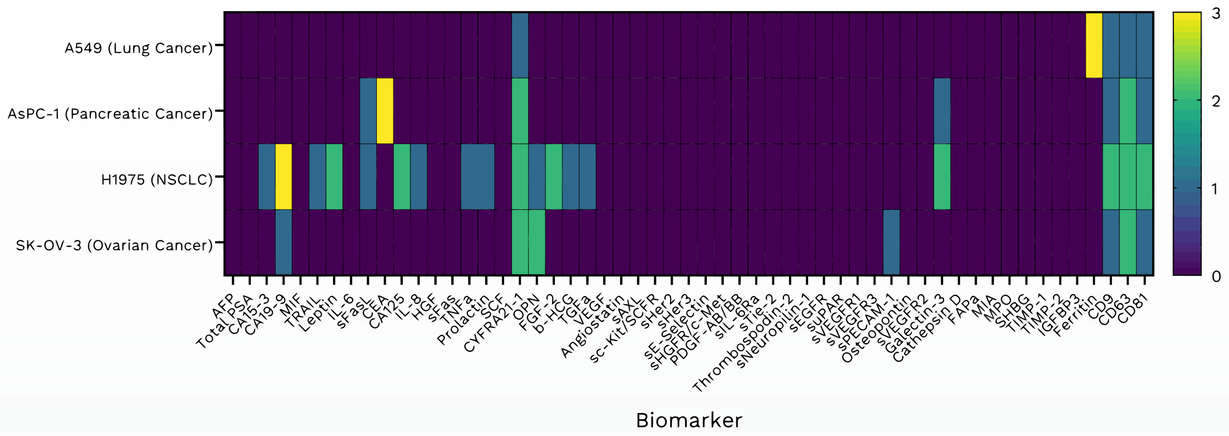
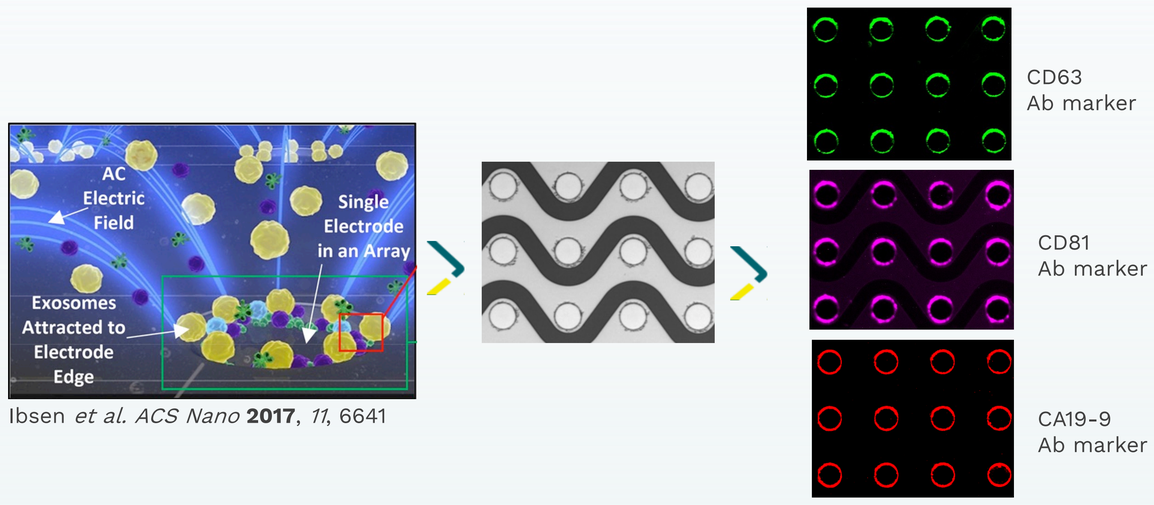


Figure 3. Heatmap of positive markers for each cell line. Standardized depiction of markers that show a positive signal, defined as the marker scaling with particle concentration. 0 means no marker expression while 3 means strong marker expression.

AC Electrokinetics (ACE) Platform



Ibsen *et al. ACS Nano* **2017**, *11*, 6641

Figure 4. Overview of ACE Microelectrode Array Platform A set of interdigitated microelectrode arrays are fabricated on a silicon chip. EVs are isolated on the circular electrodes and held in place for analysis of different types of markers, via fluorescent antibody tagging.

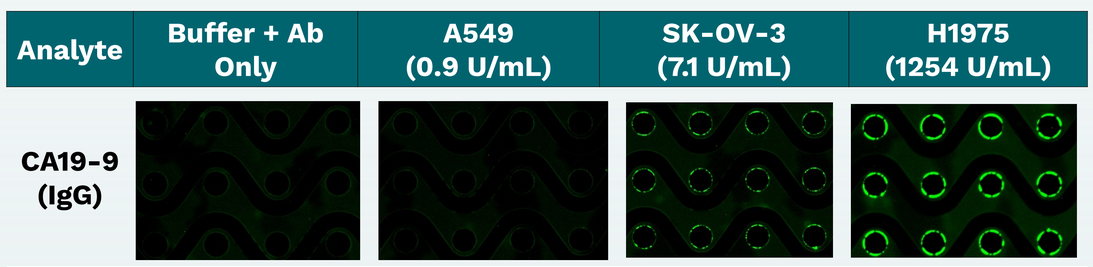


Figure 5. Detection of CA19-9 In-Situ. Images of EVs isolated on the microelectrode arrays in relation to their CA19-9 marker concentration from spiked EVs in buffer. A negative control, buffer + Ab only, is included to confirm the specificity of the detection on the captured EVs.

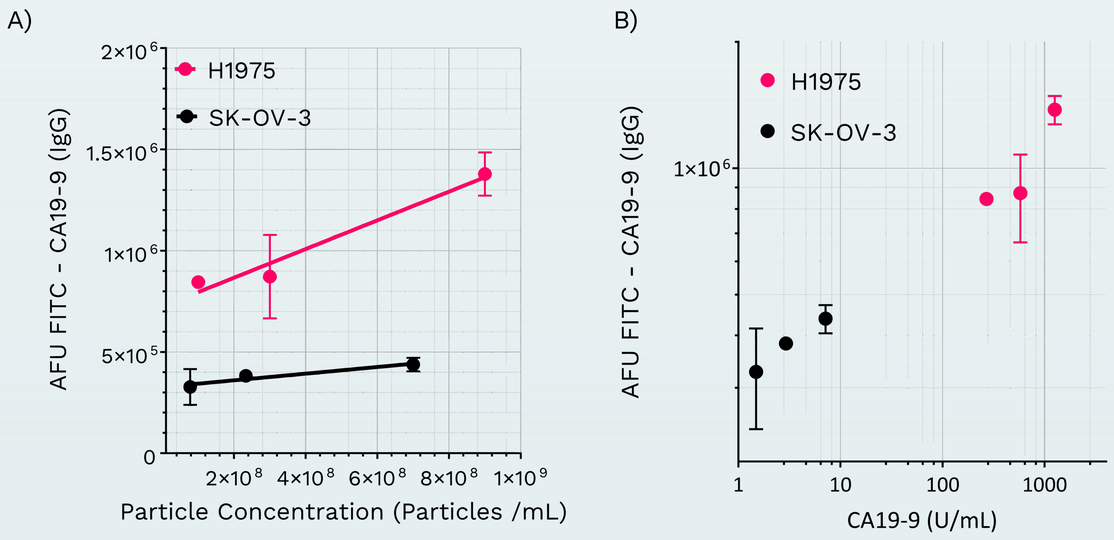


Figure 6. CA19-9 EV Marker Correlation. A) To particle concentration. B) To CA19-9 values read on commercial immunoassay. The linear correlation shown for the cell lines H1975 and SK-OV-3 suggest the location of the CA19-9 marker on the EVs. The cell lines A549 and AsPC-1 have negligible expression of CA19-9.

Conclusions

- ✓EVs hold strong potential for early cancer detection, with real-world application now within reach.
- ✓Direct linkage between EVs and cancer biomarkers strengthens the EVs diagnostic relevance.
- ✓Accessible biomarker information from EVs can enable the development of reliable, near-patient diagnostic tools.
- ✓This study illustrates specific correlations between EV markers and cancer biomarkers across various cell lines.
- ✓Findings demonstrate feasibility of simple, direct detection using EV-based approaches.