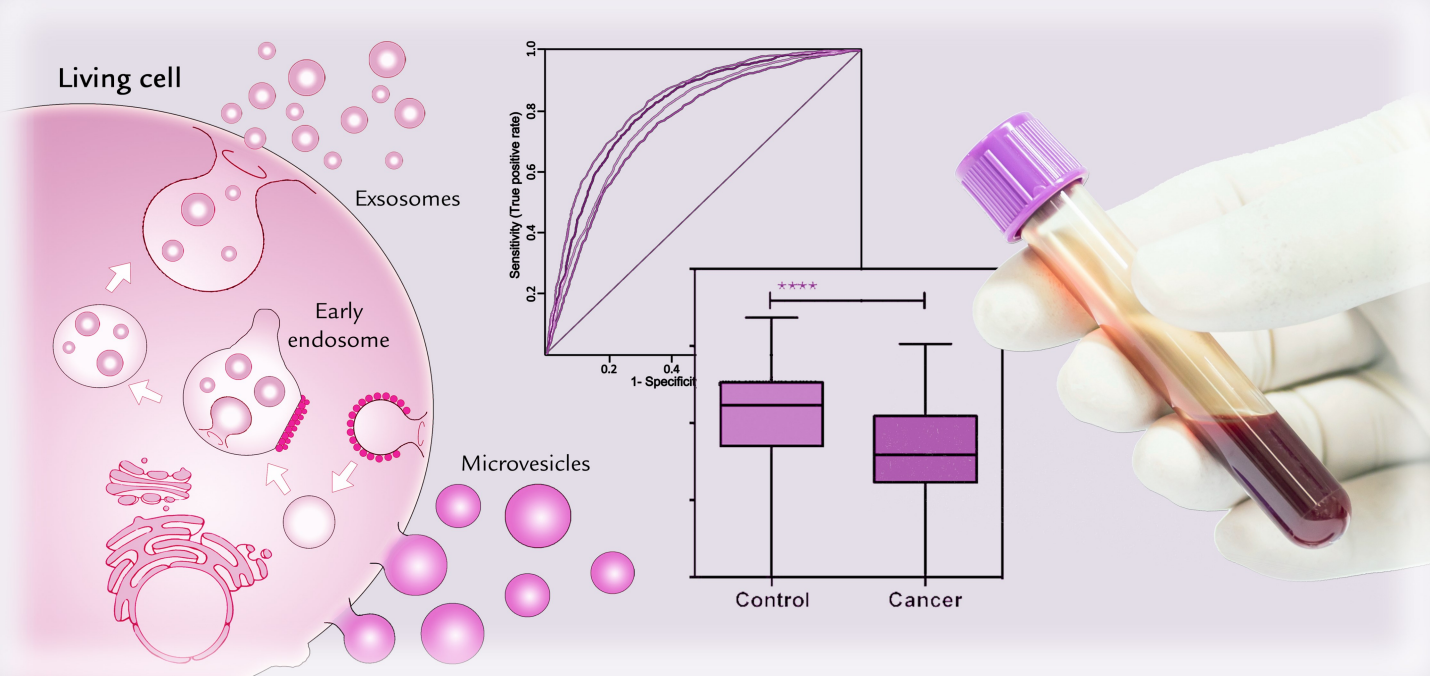
AALBORG UNIVERSITY HOSPITAL

EV ARRAY

High-throughput multiplexed phenotyping of extracellular vesicles (EVs)



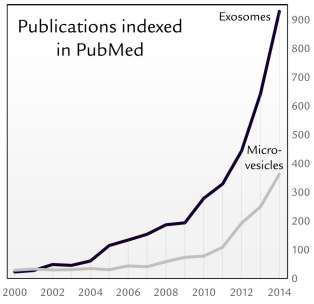
Need and novelty value

All living cells produce extracellular vesicles (EVs), which are **nano-sized compartments**. They are considered as a pivotal part of the intercellular environment and act as important players in cell-to-cell communication. The fact, that EVs are involved in the development and progression of several diseases, has formed the basis for the use of EV analyses in a **clinical setting** and envisions a great potential for using EVs as **disease-related biomarkers.**
EVs are a heterogeneous population of membrane-enclosed vesicles that can be divided into a number of subpopulations based on specific characteristics such as size, biogenesis, cellular origin, protein composition, and biological function. The two major subtypes of EVs are exosomes and microvesicles.

The research field and interest in EVs have increased tremendously during the past 5 years. The number of **indexed publications** in Pubmed are increasing almost exponentially (see graph).

The increasing interest in EVs as biomarkers and in EVs in general is demonstrated by:

- **Formation of several societies:** International Society of Extracellular Vesicles (ISEV), formed in 2011; American Society of Exosomes and Microvesicles (ASEM); United Kingdom Extracellular Vesicle Forum (UKEV Forum).
- **International journals adressed for extracellular vesicles:** Journal of Extracellular Vesicles (JEV); Exosomes and Microvesicles (ASEM).
- **National Institute of Health (NIH)** has invested more than $33 Mio. in EV research since 2013.
- **Formation of international research databases:** EVPedia, ExoCarta, VesiclePedia.



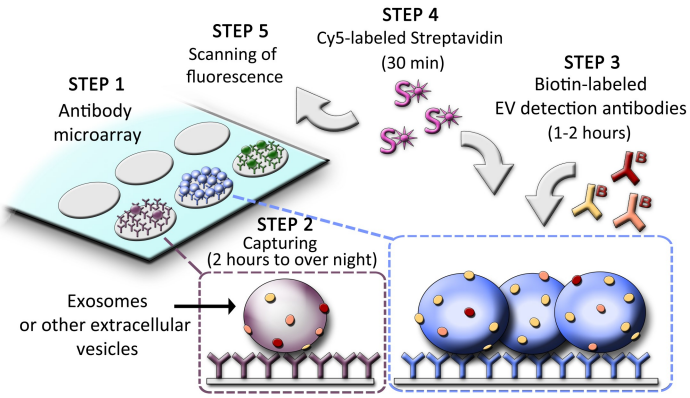


Illustration: Principle of the Extracellular Vesicle Array (EV Array)

Step 1: The EV Array is composed of different capture antibodies printed on a microarray slide. **Step 2**: 10–100 µL plasma or other body fluids (urine, saliva, BALF, etc.) are applied in a 96-well setup and incubated 2 hours to overnight. **Step 3**: The EVs are detected with a cocktail of biotinylated antibodies. **Step 4/5**: The presence and thereby phenotype of EVs is visualized after incubation with Cy5-labeled Streptavidin using a fluorescence scanner.

PARTNERSHIP

We are looking for:
(1) a partner for the ongoing development and optimization of the EV Array.
(2) a partner to make the EV Array commercial available in research and eventually clinical relations.

We will provide:
(1) Know-how and technical expertise in setting up the platform.
(2) We will be serving as a joint testing facility.

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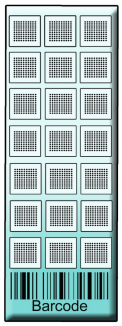
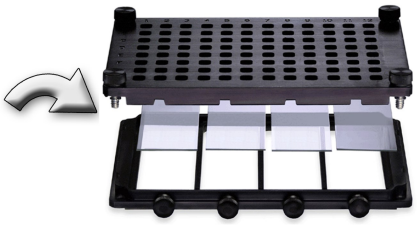
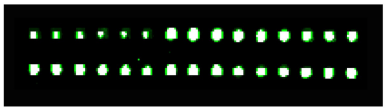
A Glass slide printed with antibodies



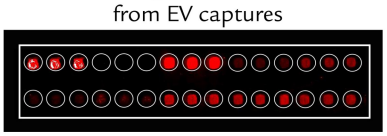
B High-throughput format (84 samples/96-well format)



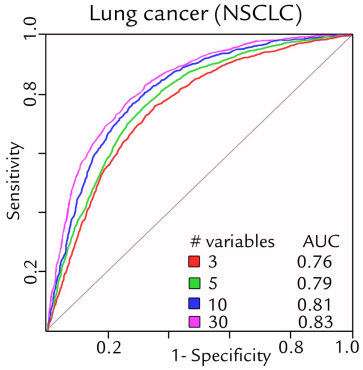
C Procedure includes preanalytical quality control



D Scanning of fluorescence from EV captures



E Diagnostic power of EVs Lung cancer (NSCLC)



Competitive advantages

The research field of EVs has shown a great demand for commercial available technologies to analyse EVs for biomarkers. Currently, extensive and time consuming (> 24 hours) purification procedures is needed prior to analysis of single markers. **No other** existing technology phenotypes EVs in a multiplexed manner using unpurified material.

The EV Array consumes only 10 – 100 µL sample, whereas purification procedures uses several milliliters. The EV Array is performed in multi-well cassettes in a high throughput manner (up to 21 samples per slide), but is still easy to handle in a laboratory.

The use of microarray as a platform with spots of 1 nL volumes minimizes the cost of the EV Array as only small amounts of antibodies are needed.

The EV Array has been tested and optimized for various bodyfluids (plasma/serum, saliva, urine, ascites, sputum, bronchoalveolar lavage (BAL), ceresponial fluid, bone marrow, synovial fluid) together with cell culture supernatants.

Current stage of technology

The development of the EV Array started in 2011 and was published in Journal of Extracellular Vesicles (JEV) in 2013. In the developmental process all steps in the procedure were optimized including the microarray production and preanalytical quality control. Slides with various coatings has been tested together with buffer systems for printing, blocking and dilution as well as sample volumes, antibody concentrations, incubation time and temperature.

The current stage of the EV Array technology is a fully developed analysis optimized for a small-to-medium laboratory research scale.

To make the EV Array commercial available, a larger production of microarray slides including quality control is needed. Various commercial product combinations should be considered; predefined arrays or special customized arrays; kits (microarray slides, antibody solutions, buffers, cassettes); and additional services (fluorescence scanning and data treatment).

Illustration: The EV Array in practice

A) Barcoded microarray glass slides (7.5 x 2.5cm) printed in a 21-well setup. **B)** Printed slides are placed in multiwell-cassettes for either one glass slide (up to 21 samples) or four glass slides (up to 84 samples). **C)** The procedure is validated by a quality control of the printed spots, which includes positive and negative controls. **D)** Scanning after capture and detection of EVs can be performed on a normal laboratory fluorescence scanner or on a specialized microarray scanner for maximum sensitivity. **E)** The diagnostic power of the EVs to identify lung cancer patients can be visualized by the receiver operating characteristics (ROC), which shows the models' sensitivity, specificity and accuracy. Data presented was obtained by analyzing EVs from 10 µL of plasma from 109 lung cancer patients (NSCLC) together with 110 lung diseased patients (non-cancerous). Thirty-seven different EVs markers were analyzed simultaneously and a multivariate data analysis was performed. Using the results from 30 of the variables it was possible to distinguish lung cancer patients from other lung diseased patients with a specificity of 0.79 and a sensitivity of 0.73 with an accuracy of 76%.

INVENTORS

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Publication references

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