Ballroom F-H
Symposium session 4C - EV proteomics and lipidomics
Chairs: *Hidetoshi Tahara and An Hendrix* 14:00-15:30

O-4C-1

In-depth proteomics analysis of human breast milk-derived extracellular vesicles to reveal their origin and targets in the infant's developing immune system

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Introduction: Besides providing nutrition, breast milk delivers important signals that stimulate the infant's developing immune system. It has been postulated that extracellular vesicles (EV) in milk support the instruction and/or development of neonatal immunity. However, little is known about the composition of milk-derived EV, partly due to the difficulty to purify EV from other components in milk. *Methods*: In this study, an extensive LC-MS/MS proteomic analysis was performed, whereby EV were isolated from breast milk of 7 individual donors using our recently established optimized density gradient-based isolation protocol [1]. High-density, non-floating complexes were included to compare the contents of EV to other macromolecular structures in milk. A comprehensive protein network was composed tracing the possible cellular origins of milk-derived EV and the potential targets in the gut. *Results*: An average of 579 proteins was identified in EV, compared to 205 proteins in the non-floating fraction. Interestingly, EV associated proteins like ANXA5 and Flotillin were exclusively identified in EV, while CD9, CD63 and CD81 were also present in non-floating protein complexes. Additionally, MHC-II was identified in the EV fraction only, suggesting that antigenic epitopes may be delivered via EV released from antigen-presenting cells. Besides MHC-I, the mammary epithelial cell marker beta-1,4-galactosyltransferase (lactose subunit) was identified in the EV fraction only, demonstrating EV of epithelial origin. Furthermore, several adhesion molecules (ICAM-1, CEACAM-1) were associated to EV which could allow EV binding to gut epithelial cells and gut resident immune cells. *Summary/conclusion*: In-depth proteomic analysis and compilation of an extensive network of EV proteins involved in immunity demonstrates that milk-derived EV originate from multiple cellular sources and have the ability to target various cell types in the gut.

O-4C-2

Verification of predictive biomarker candidates of colorectal cancer metastasis in serum extracellular vesicles by selected reaction monitoring-based targeted proteomics

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Introduction: Recently, the release of extracellular vesicles such as exosomes into biological fluids calls attention to their promise as circulating biomarkers in the surveillance of disease state such as cancer progression. Since proteins in the extracellular vesicles are very stable, they are the ideal resource for proteomics based biomarker discovery. In addition, high abundance serum/plasma proteins can be removed during isolation process of the extracellular vesicles, which can eliminate a huge potential source of interference in mass spectrometric analysis. *Methods*: A quantitative proteomic analysis for biomarker discovery was performed by iTRAQ labelling using membrane protein of colorectal cancer tissue. Serum extracellular vesicles were prepared by ultracentrifugation. The biomarker candidates identified in colorectal cancer tissue were verified in serum extracellular vesicles obtained from health controls (n = 20), colorectal cancer patients with and without metastasis (n = 18 each) by SRM/MRM targeted proteomic approach using stable isotope-labelled reference peptides. *Results*: We have previously identified and verified biomarker candidates of colorectal cancer in membrane fractions of colorectal cancer tissues using iTRAQ and SRM/MRM methods. Among about 5,500 proteins identified, 105 membrane and extracellular proteins were shown to be differentially expressed between adenomas, cancer without metastasis and with metastasis. These biomarker candidates were verified by SRM/MRM using stable synthetic isotope-labelled peptides as an internal control (Kume et al., Mol Cell Proteomics 13: 1471–84, 2014). Then, we investigated if we can detect and quantify the biomarker candidates in the extracellular vesicles containing fraction of serum by SRM/MRM. Among 100 biomarker candidates, we could quantitate more than 20 proteins in the extracellular vesicles containing fraction of serum. Moreover, we were able to verify 3 predictive biomarker candidates for colorectal cancer metastasis by SRM/MRM. Intriguingly, genes corresponding to the 3 biomarker candidates in extracellular vesicles have been reported to reside in 19q13 region that is amplified in advanced colorectal cancer. Further validation of the predictive biomarker candidates are currently under investigation. *Summary/conclusion*: These results suggest that targeted proteomic technology is a powerful tool to identify and verify novel promising biomarkers for diagnosis in the extracellular vesicles of biological fluids.

O-4C-3

Selection of extracellular vesicle subsets markers by proteomics

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Introduction: Extracellular vesicles (EV) are a heterogeneous population of nano-sized vesicles with important regulatory roles. EV attributes depend on the type and status of the cell secreting them. We hypothesize that the targets and functions of EVs are determined by their constituent protein, lipid, and RNA components, and that EV subsets produced by different cells (and under different conditions) have functional differences that are due to and identifiable by their distinctive component repertoires. Although certain proteins (such as CD9, TSG101, and Alix) are recognized as common components among EVs, we hypothesize that subset-specific components contribute to subset-specific functions. In order to determine whether EV subsets are relevant in performing regulatory effects, we must be able to isolate EV subsets from the bulk population of EVs and