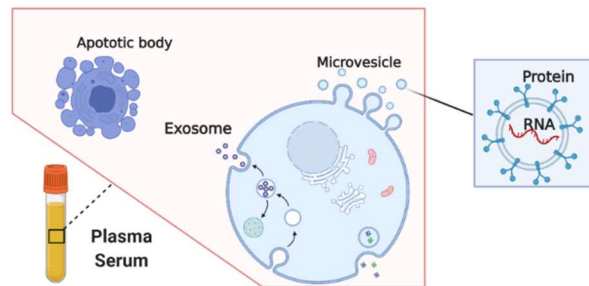




## Tissue-specific exosome marker candidates have been identified from blood for the development of highly accurate next-generation biomarkers.

- More than 4000 proteins contained in exosomes in healthy human blood (serum and plasma) were identified and published as a catalogue.
- Using this catalogue, candidate marker proteins were identified from blood exosomes as markers of exosomes secreted from specific tissues.
- The exosomal protein information obtained from healthy individuals in the catalogue is expected to be useful as reference data not only for the diagnosis and monitoring of pathological conditions of various diseases, including cancer and dementia, but also for health management at the stage of unwellness.



Schematic diagram of proteins contained in extracellular vesicles including exosomes in blood.

### 【Abstract】

Exosomes (extracellular vesicles)\*1 contain secreted intracellular proteins and nucleic acids. Exosomes secreted into the bloodstream have therefore attracted much attention in recent years in the development of biomarkers\*2 for the early diagnosis of diseases and monitoring of pathological conditions. Diagnosis using blood exosomes is minimally invasive, allowing for continuous observation and likely to immediately detect pathological changes caused by disease.

A research group of Jun Adachi, project leader of the Laboratory of Proteomics for Drug Discovery and Laboratory of Clinical and Analytical Chemistry at NIBIOHN, and Dr Satoshi Nagayama, surgeon at Uji Tokushukai Hospital, published a catalogue of proteins contained in exosomes secreted in the blood in the journal *iScience*. Furthermore, the catalogue was used to identify tissue-specific exosome marker candidates that can be used to enrich tissue-specific exosomes.

By utilizing the exosomal protein information in the catalogue as basic data for healthy individuals, it is expected to be useful not only for the diagnosis of various diseases, including cancer and dementia, but also for health management at the stage of unwellness.

## ❖ Background

Exosomes secreted by cells into the blood contain intracellular substances such as proteins and nucleic acids and are thought to reflect the characteristics of the cell from which they are secreted. In addition, they are stable in the blood and are expected to be a source of information for the development of clinical biomarkers.

In 2017, our research group discovered a high-performance colorectal cancer biomarker protein from blood exosomes\*3. This time, using the cutting edge exosome analysis technology, we decided to compile a catalogue of proteins contained in exosomes in the blood of healthy people.

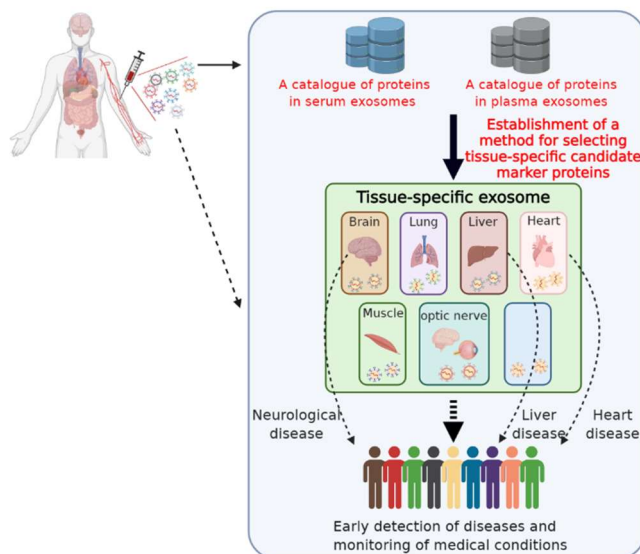
Blood contains a mixture of exosomes secreted from nearly all tissues and cells, and exosomes from other tissues can mask changes caused by disease, making it difficult to detect the presence of biomarkers that reflect disease. This problem is more pronounced in the early stages of disease and hinders the development of needed early diagnostic markers. If exosomes derived from the tissue of interest could be purified, it would be possible to observe changes in exosomes secreted from the site of disease with a high degree of accuracy, even in the early stages of the disease. In view of the above, an attempt was made to identify proteins that could serve as markers to distinguish between exosomes derived from different secretory tissues.

## ❖ Results

Exosomes were purified from serum and plasma samples from healthy subjects, and through quantitative proteome analysis\*4, a catalogue of proteins in blood exosomes was successfully compiled on a scale of over 4,000 proteins, far exceeding previous studies, and published in iScience. The data on signal intensities and inter-individual differences for each protein in serum and plasma exosome in this catalogue can also be used for next-generation healthcare\*5. Our study also identified several new marker proteins that show less variation between individuals compared to known markers and are expected to be used as novel standard markers.

Furthermore, by combining this catalogue with information from a public database that defines tissue-specific proteins (Human protein atlas, HPA\*6), we found that a number of tissue-specific proteins are also contained in blood exosomes.

For example, a group of brain tissue-specific proteins were identified in exosomes and these proteins were found to show similar patterns of variation between individuals by co-regulation analysis\*7. Network analysis has revealed a number of proteins that have been reported to be associated with neurodegenerative diseases, including amyloid precursor protein (APP), microtubule-associated protein tau (MAPT), presenilin 1 (PSEN1) and huntingtin (HTT), suggesting that each of these proteins interact with each other and are present in exosomes. Therefore, if a technology to purify brain-derived exosomes will be established in the future, it is expected to be applied as a new brain monitoring technology to diagnose and monitor the pathology of neurodegenerative diseases such as Alzheimer's disease, progressive supranuclear palsy and Huntington's disease.



Protein catalogue of blood-secreted exosomes and method of selecting tissue-specific marker candidate proteins.

#### ❖ Future Outlook

The catalogue of exosomal proteins in healthy human blood produced in our study can be used as reference data for disease analysis. It is therefore expected to contribute to a wide range of clinical biomarker studies from the development of novel biomarkers for various diseases to clinical applications. In addition, if exosomes secreted into the blood from each tissue can be purified to a high purity using the marker proteins identified in this study, it will lead to the discovery of diagnostic biomarkers with higher sensitivity and specificity.

In view of the above, the results of this research are expected to contribute to a wide range of fields, from clinical applications in early diagnosis and monitoring of pathological changes in various diseases such as cancer, neurological diseases, and infectious diseases, to basic research such as elucidation of pathological mechanisms and health management at the stage of unwellness.

#### ❖ Remarks

This research was published in the online edition of *iScience* on 1<sup>st</sup>, March 2022.

Comprehensive proteomic profiling of plasma and serum phosphatidylserine-positive extracellular vesicles reveals tissue-specific proteins in healthy individuals. Satoshi Muraoka, Masayo Hirano, Junko Isoyama, Satoshi Nagayama, Takeshi Tomonaga, Jun Adachi. *iScience*, in press.

DOI 10.1016/j.isci.2022.104012 ( <https://linkinghub.elsevier.com/retrieve/pii/S2589004222002826> )

The study was partially supported by the Public/Private R&D Investment Strategic Expansion Program (PRISM).

## ❖ Glossary

### ※1: Exosomes (extracellular vesicles)

Membrane vesicles surrounded by a lipid bilayer that are secreted by almost all cell types. They are attracting attention as a new intercellular signaling medium, carrying proteins, nucleic acids (microRNA, messenger RNA, DNA, etc.), lipids and metabolites from the parent cell. The development of biomarkers for diagnosis and therapy is rapidly developing. Exosomes<sup>™</sup> are part of a population of extracellular vesicles, but in this article extracellular vesicles are described as exosomes.

### ※2: Biomarker

Indicators of biological information for quantifying and quantifying changes in a substance in vivo. It is expected to be used not only for early detection of diseases and monitoring of medical conditions, but also for various applications such as prediction of adverse effects and prevention of diseases.

### ※3: Quantitation of putative colorectal cancer biomarker candidates in serum extracellular vesicles by targeted proteomics.

Shiromizu T, Kume H, Ishida M, Adachi J, Kano M, Matsubara H, Tomonaga T.

Sci Rep. 2017 Oct 6;7(1):12782. DOI 10.1038/s41598-017-13092-x

### ※4: Quantitative proteome analysis

An analytical method for quantifying the amount of protein expression in a sample in a single, comprehensive measurement.

### ※5: Next generation healthcare

A system that combines a vast amount of data on the human body, from its physiological functions to behavior, with artificial intelligence (AI) and other cutting-edge IT technologies to enable individuals to receive the necessary prevention, pre-symptomatic care, treatment, and nursing care at the right time.

### ※6: Human protein atlas, HPA

Database of information on protein expression and localization in various human organs, tissues and cells provided by a Swedish-based research team.

### ※7: co-regulation analysis

This is a method of analyzing the relationship between two variables in terms of how they both vary. This makes it possible to analyze the relationship between the two proteins.