Press Release

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Keio University School of Medicine
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JVCKENWOOD Corporation
Japan Agency for Medical Research and Development

Development of a Highly Sensitive Device for Counting the Disease-Specific Exosomes in Cancer Patient Sera by Combining Properties of New Optical Disc and Nano-Bead Technologies

A research team at Keio University School of Medicine have developed ExoCounter, an innovative exosome measurement system[1] that integrates optical disc and nano-bead technologies[2] The team was led by Assistant Professor Yasuaki Kabe, Department of Biochemistry, in a joint study with Professor Hiroshi Handa, MD and PhD, at Department of Nanoparticle Translational Research, Tokyo Medical University, and JVCKENWOOD Corporation.

ExoCounter is a new measurement system that allows for precise and easy measurement of the number of disease-specific exosomes. The system binds magnetic nano-beads to disease-specific exosome proteins (surface antigens) on a special optical disc, which is then used to detect the exosome-nanobead complex using an optical disc drive. Exosomes exist mainly in blood and are a type of small particle that is secreted from various cells. ExoCounter renders previously required pretreatments such as exosome purification unnecessary for detecting cancer-specific exosomes secreted from cancer cells and is likely to lead to advances in cancer diagnoses and other applications.

In this study, a large-scale disease cohort study using specimens from the BioBank Japan Project of the Japan Agency for Medical Research and Development (AMED), researchers used an optical disc surface to bind magnetic nano-beads and exosomes that contained HER2 surface antigens secreted from cancer cells, detected the exosome-nanobead complex using ExoCounter, and measured the number of exosomes generated due to cancer. This revealed, for the first time, that the sera of breast and ovarian cancer patients statistically contain a significantly high number of cancer-specific exosomes in which HER2, a known cancer marker, is expressed.

These results are likely to lead to further developments in cancer research, including new cancer diagnoses and cancer treatment methods using exosomes as an indicator.

The study results were published in an early online release of the US scientific journal *Clinical Chemistry* on Wednesday, July 18, 2018.

1. Research Background and Outline of the study

Exosomes, cell-secreted membrane vesicles only 50 to 150 nm in diameter, play an important role in regulating physiological processes by transferring genetic information of diverse mRNAs and microRNAs, as well as proteins, from cell to cell. Experts have increasingly expected that exosomes could be a potentially useful biomarker, given that many studies have suggested that the types and quantities of exosomes in blood are related to cancer conditions and subsequent progress because exosomes are also closely linked to cancer metastasis. With this in mind, experts have been actively conducting studies on exosomes using existing measurement techniques designed to measure particles and cells. However, given that exosomes are extremely small and blood contains a significant variety of particles comparable to exosomes in size, a complicated purification pretreatment was necessary to detect exosomes, which presented a challenge for detecting exosomes with high precision. In particular, it was difficult to accurately and easily measure the number of cancer-specific exosomes in blood.

In this study, the research team developed ExoCounter, an exosome measurement system that allows for quantitative measurements of cancer-specific exosomes in sera accurately and easily without performing any pretreatment. This new method integrates JVCKENWOOD optical disc technologies and FG Beads, a patented nano-magnetic bead technology developed by Keio University School of Medicine and Tokyo Medical University. The study demonstrated the functionality of ExoCounter by measuring cancer-specific exosomes released from cancer cells and cancer patient sera.

2. Research Significance and Future Development

ExoCounter captures exosomes on an optical disc surface before binding them with FG beads, which puts the exosomes into a state in which they can be detected on an optical spot. The number of exosomes are then measured by detecting FG beads bound to exosomes using a newly-developed optical disc drive (See Fig. 1).

The optical disc surface has a nanostructure specially tailored to the sizes of the exosomes, and by coating the surface with an antibody capable of binding to exosomes, it is possible to selectively capture exosomes in sera and other biological samples. In addition, cancer-specific exosomes can be detected by binding these exosomes FG beads coated with cancer-specific antibodies.

For practical verification, the researchers used ExoCounter to measure exosomes isolated by ultracentrifugation from a colorectal cancer cell culture. This resulted in exosomes being detected only when using exosome-specific antibodies, which showed that ExoCounter is able to detect exosomes with high selectivity. In addition, when a sample with an altered exosome concentration was measured, the number of exosomes was found to be proportional to the altered concentration (See Fig. 2A). Similarly, the number of exosomes found in sera samples was also selectively detected in proportion to the amount of sera.

In order to verify the potential for measuring cancer-specific exosomes, the research team measured the number of exosomes in cultures of colorectal cancer, breast cancer, and lung cancer cells using FG beads containing immobilized antibodies that bind to cancer-related proteins. This showed that exosomes carrying the cancer-specific HER2 protein on their surface can be measured with high accuracy using ExoCounter (See Fig. 2B).

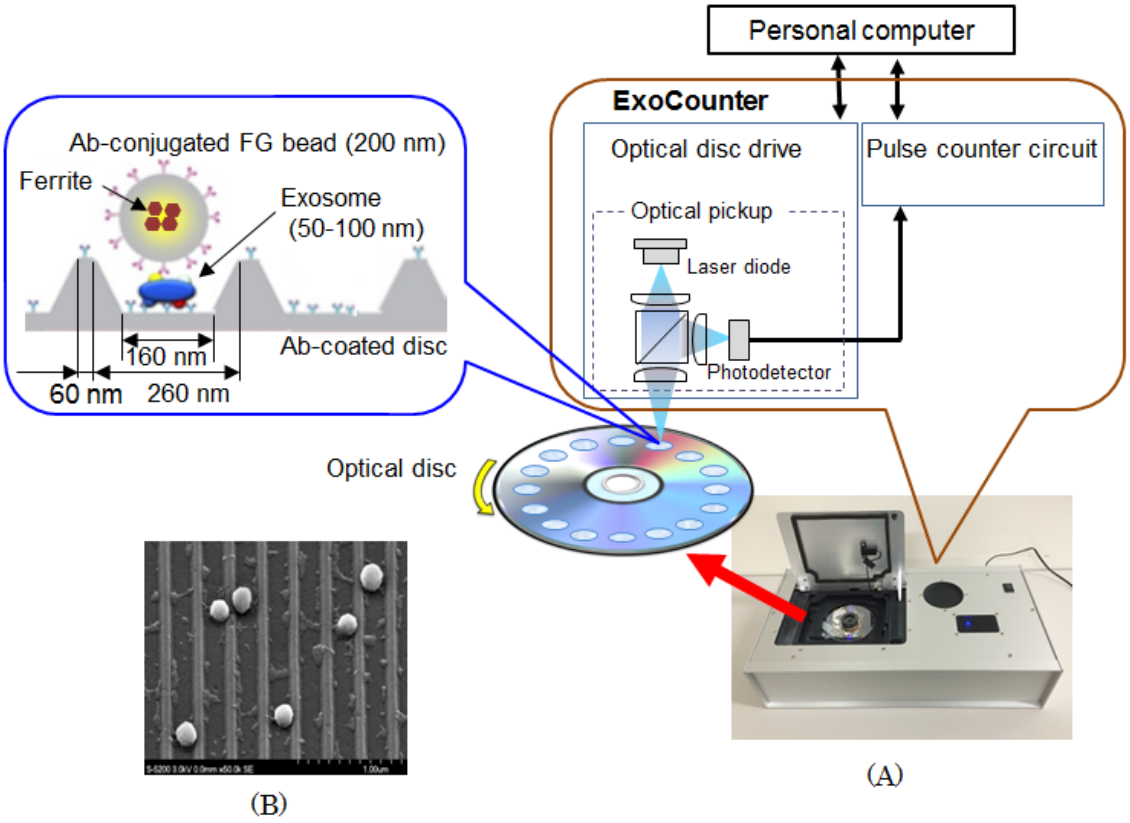

Fig. 1 (A) Exosome measurement system ExoCounter (B) Optical disc surface as observed with an electron microscope. Exosomes captured on the optical disc are bound to magnetic nano-beads (FG beads).

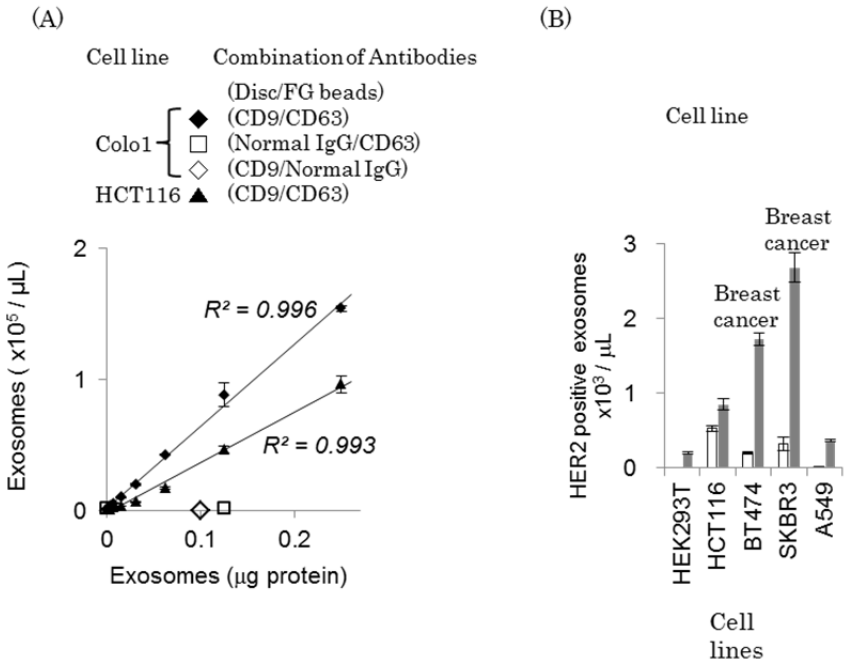

Fig. 2
(A) Symbols □ and ◇indicate the use of non-exosome binding antibodies, while symbols ◆and ▲indicate the use of exosome binding antibodies.
When using non-exosome binding antibodies, the number of exosomes counted was close to 0, but when using exosome binding antibodies, the number of exosomes could be detected in proportion to the exosome concentration.
(B) Measurement of various cell cultures. The concentration of exosomes carrying the cancer-specific HER2 protein was shown to be particularly high in the breast cancer cell cultures (BT474, SKBR3). The white bars show the results when using antibodies not specific to exosomes.

Based on these results, the research group used ExoCounter to measure exosomes in the sera of both cancer and non-cancer patients collected in the Biobank Japan Project as well as the sera of healthy subjects collected by Tohoku University Tohoku Megabank Organization. The results verified that the sera of breast and ovarian cancer patients contain a significantly higher number of exosomes carrying the cancer-specific HER2 protein in comparison to the sera of healthy persons or patients with non-cancerous diseases (See Fig. 3). Although there have been qualitative studies to date that report a higher number of HER2 carrying exosomes in cancer patient specimens, this large-scale cohort study shows, for the first time, that cancer patient sera contain a statistically significant high number of HER2-carrying exosomes.

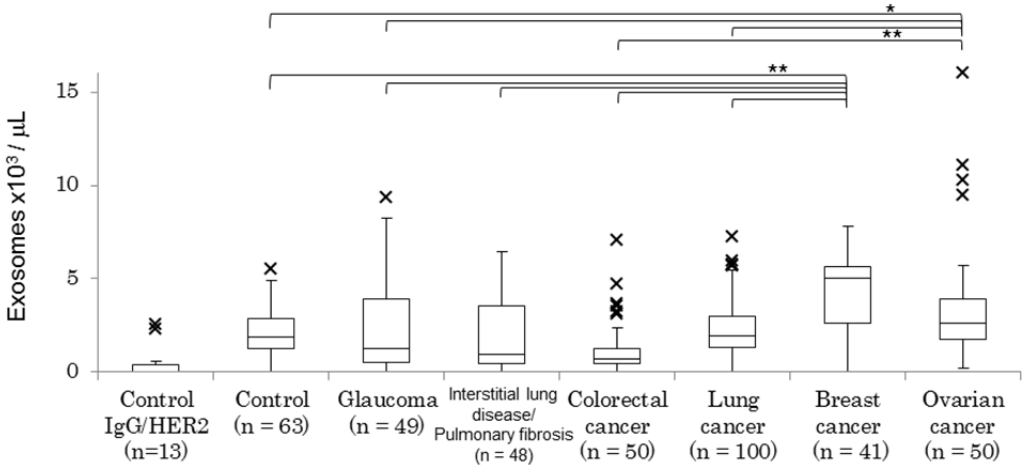

Fig. 3
Measurement of cancer-specific exosomes in the sera of cancer patients, non-cancer patients, and healthy subjects. Median results were high for both breast and ovarian cancer patients. A statistical analysis performed on these two sample groups showed that the sera of these patients contain a significantly high number of cancer-specific exosomes in comparison to the sera of healthy subjects and non-cancer patients. ("n" denotes the number of specimens and "×" denotes the number of data items not within the framework. Symbols "★★" and "★" denote 1% and 5%, levels of significant difference in statistical analysis.)

ExoCounter is an innovative measurement system that allows for quick and easy measurement of the number of exosomes in a body fluid specimen such as sera and could potentially be an important diagnostics technique for liquid biopsy[3], and is already being offered by JVCKENWOOD as a research measurement system. ExoCounter also has potential application as a useful tool for analyzing disease-specific exosomes related to cancer and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, as well as autoimmune diseases, inflammatory diseases, and depression, among others. It is also expected to play a major role in developing new exosome-based diagnostics techniques.

3. Notes

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4. Research Paper

Title: Development of a Highly Sensitive Device for Counting the Number of Disease-Specific Exosomes in Human Sera

Japanese Title: ヒト血清中の疾患特異的エクソソームの数を計測するための高感度デバイスの開発

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[Glossary]
Note 1 - JVCKENWOOD Website: http://healthcare.jvc.com/exosome/exocounter/
Note 2 - Nano-bead: A type of bead that is on the order of one nanometer in length (nm or one millionths of a millimeter). The technique in question uses nano-beads containing ferrites approximately 200 nm in diameter. The bead surface is coated with antibodies that bind with proteins on exosomes, which singles out target exosomes for detection.
Note 3 - Liquid biopsy: A technique often used in the field of oncology to diagnose and predict treatment effects using non-solid biological tissue samples such as blood as opposed to conventional biopsies, which extract tumor tissue samples using an endoscope or needle.

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