News Release

Development of a Novel Liquid Biopsy Method Using Extracellular Vesicle-derived DNA from Ovarian Cancer Patients

Key Points

•The amount of extracellular vesicle-derived DNA, or EV-DNA, was significantly increased in ascites from patients with ovarian cancer compared with that from patients with benign ovarian tumors.

•EV-DNA levels were elevated even in patients with ovarian cancer whose ascites cytology was negative, suggesting the potential of EV-DNA as a biomarker to support the diagnosis of ovarian cancer.

•Copy number variation, or CNV, profiles of ascites-derived EV-DNA closely reflected CNV profiles of tumor tissue DNA.

•Targeted CNV analysis using droplet digital PCR enabled prediction of therapeutic response to the PARP inhibitor olaparib.

•Ascites-derived EV-DNA may be useful as a minimally invasive liquid biopsy approach for diagnostic support and personalized treatment in ovarian cancer.

Summary

A research group led by Dr. Ryosuke Uekusa of the Department of Obstetrics and Gynecology, Nagoya University Graduate School of Medicine, currently Section Chief of Gynecology at Aichi Cancer Center; Dr. Akira Yokoi, Lecturer at Nagoya University Hospital and also affiliated with the Nagoya University Institute for Advanced Research; and Professor Hiroaki Kajiyama of Nagoya University Graduate School of Medicine has developed a novel liquid biopsy method that may be useful for diagnostic support and prediction of therapeutic response in ovarian cancer by analyzing extracellular vesicle-derived DNA, or EV-DNA, present in ascites from patients with high-grade serous ovarian carcinoma, or HGSOC.

Ovarian cancer is one of the most lethal gynecologic malignancies and is often diagnosed at an advanced stage. In particular, HGSOC frequently shows not only gene mutations but also genome-wide copy number variations, or CNVs. However, in some patients, sufficient tumor tissue cannot be obtained, and existing diagnostic methods may not provide enough information for diagnosis or treatment selection. Therefore, new biomarkers that can obtain

tumor-related information from body fluids in a minimally invasive manner are needed.

In this study, the researchers isolated extracellular vesicles, or EVs, from ascites samples obtained from patients with ovarian cancer and benign ovarian tumors, and analyzed the amount of EV-DNA and its CNV profiles. They found that EV-DNA levels were significantly increased in ascites from patients with ovarian cancer. Furthermore, EV-DNA levels were elevated even in patients with ovarian cancer whose ascites cytology was negative, suggesting that ascites-derived EV-DNA may be a useful biomarker to support the diagnosis of ovarian cancer.

The researchers also found that CNV profiles of ascites-derived EV-DNA accurately reflected those of tumor tissue DNA. Compared with conventional cell-free DNA, EV-DNA contained more abundant tumor-related genomic information. In addition, targeted gene analysis using droplet digital PCR, or ddPCR, identified a five-gene CNV signature consisting of *ARID1A, NOTCH3, CSMD3, ELP4*, and *BARD1* that predicted response to the PARP inhibitor olaparib with high accuracy.

These findings suggest that ascites-derived EV-DNA may contribute to diagnostic support and personalized treatment in ovarian cancer as a minimally invasive liquid biopsy platform. Future prospective studies with larger patient cohorts are planned to further validate the clinical utility of this method.

Research Background

Ovarian cancer is one of the gynecologic malignancies with the poorest prognosis. Because it often causes few subjective symptoms in the early stages, many patients are diagnosed only after the disease has already progressed. High-grade serous ovarian carcinoma, or HGSOC, is the most common histological subtype of ovarian cancer and is associated with a high recurrence rate. Therefore, the development of new diagnostic methods and biomarkers that predict treatment response remains an important clinical challenge.

In recent years, PARP inhibitors have been widely used for the treatment of ovarian cancer. Biomarkers such as BRCA gene mutations and homologous recombination deficiency, or HRD, are currently used for treatment selection. However, these existing biomarkers do not always fully predict actual treatment

response in clinical practice. Thus, new biomarkers that more accurately reflect therapeutic sensitivity are needed.

In addition, tumor tissue cannot always be easily obtained from patients with ovarian cancer, particularly in patients for whom surgery is difficult or tissue sampling is limited. For this reason, liquid biopsy technologies that obtain tumor information from body fluids, such as blood and ascites, are expected to provide minimally invasive alternatives.

Extracellular vesicles, or EVs, are small membrane-bound particles secreted by cells. They contain various molecules, including DNA, RNA, and proteins, and are stably present in many body fluids, including blood and ascites. Therefore, EVs have attracted attention as potential biomarkers for cancer diagnosis and treatment monitoring.

In this study, the research group focused on EV-DNA in ascites from patients with ovarian cancer and investigated whether the amount and CNV profiles of EV-DNA could be applied to diagnostic support and prediction of therapeutic response.

Research Results

The research group isolated EVs from ascites samples collected from patients with ovarian cancer and benign ovarian tumors, extracted EV-DNA, and performed molecular analyses. First, they found that EV-DNA levels were extremely low in ascites from patients with benign ovarian tumors, whereas EV-DNA levels were significantly higher in ascites from patients with ovarian cancer.

The researchers then analyzed EV-DNA levels according to ascites cytology results. EV-DNA levels were significantly higher not only in cytology-positive ovarian cancer cases but also in cytology-negative cases compared with benign disease. This finding suggests that an increase in EV-DNA may be detected even before malignant cells are clearly identified by conventional cytology, supporting the potential usefulness of EV-DNA as an auxiliary diagnostic biomarker for ovarian cancer.

Next, the researchers analyzed CNV profiles of ascites-derived EV-DNA. They found that EV-DNA CNV profiles closely reflected CNV profiles of tumor tissue

DNA. In contrast, the concordance between serum-derived EV-DNA and tumor DNA was limited.

The study also compared conventional cell-free DNA with EV-DNA and showed that EV-DNA may contain more abundant tumor-related genomic information. These results suggest that EV-DNA is a useful material for capturing tumor genomic information in ovarian cancer.

Furthermore, with future clinical application in mind, the researchers performed targeted gene analysis using droplet digital PCR, or ddPCR, which is simpler and highly sensitive compared with comprehensive sequencing approaches. As a result, they identified a CNV signature consisting of five genes—*ARID1A, NOTCH3, CSMD3, ELP4*, and *BARD1*—that predicted response to the PARP inhibitor olaparib with high accuracy.

Taken together, these findings indicate that ascites-derived EV-DNA may be applied not only as an auxiliary diagnostic biomarker for ovarian cancer but also as a novel biomarker for predicting response to PARP inhibitor therapy.

Research Summary and Future Perspective

This study demonstrated that quantitative changes and CNV profiling of EV-DNA may be useful for diagnostic support and prediction of therapeutic response in ovarian cancer.

In particular, the finding that EV-DNA levels were elevated even in patients with negative ascites cytology suggests that EV-DNA analysis may detect tumor-related information that cannot be captured by conventional cytology. In the future, this approach may be developed as a new diagnostic support method for ovarian cancer.

In addition, because EV-DNA analysis showed potential for predicting response to PARP inhibitors, it may contribute to personalized medicine by helping clinicians select the most appropriate treatment for each patient. The research group plans to validate the diagnostic and therapeutic predictive performance of this method through prospective clinical studies involving larger patient cohorts.

Furthermore, by developing technologies to recover tumor-related EVs from

blood with higher accuracy, this approach may be expanded beyond ascites to blood-based liquid biopsy, providing an even less invasive method for future clinical application.

Publication
Journal: *Journal of Extracellular Vesicles*
Title: DNA copy number profiling in extracellular vesicles as clinical biomarkers of high-grade serous ovarian carcinoma
Authors: Ryosuke Uekusa, Akira Yokoi, Hiroaki Kajiyama, et al.

DOI: 10.1002/jev2.70308

Japanese ver.
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