News Release

Title

DNA methylation panel that can diagnose and distinguish 27 types of malignant tumors at the same time was successfully generated

Key Points

- By comprehensively statistically analyzing the methylation status of about 450,000 of the cytosines on DNA, cytosine characteristically methylated in each of the 27 types of malignant tumors was extracted, and then, DNA methylation panel that can diagnose and distinguish 27 types of malignant tumors at the same time was successfully generated.
- The methylation panel could identify the cancer cell of origin using the methylation profiles of several sets of clinical tissue samples, including primary, metastatic, and multiregional cancer samples, independent of the methylation analysis platform, specimen preparation method, ethnicity and geographic background.
- The methylation panel could be applied to methylation data of circulating tumor DNA (ctDNA) samples from patients with malignant tumor.
- The methylation panel is useful in identifying the tissue origin of cancer of unknown primary.

Summary

Dr. Dai Shimizu(Assistant Professor, Department of Gastroenterological Surgery (Surgery II), Nagoya University Graduate School of Medicine (Prof. Yasuhiro Kodera)), Dr. Kenzui Taniue (Associate professor, Isotope Science Center, The University of Tokyo (Prof. Nobuyoshi Akimitsu)), Dr. Yusuke Matsui (Associate professor, Department of Integrated Health Science, Nagoya University Graduate School of Medicine), Dr. Hiroshi Haeno (Project Associate Professor, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo) and Prof. Koshi Mimori (Department of Surgery, Kyushu University Beppu Hospital) successfully generated DNA methylation panel (CACO (CAncer Cell-of-Origin) methylation panel) CACO (CAncer Cell-of-Origin) methylation panel CACO (CAncer Cell-of-Origin) methylation panel CACO (CAncer Cell-of-Origin) methylation panel that can diagnose and distinguish 27 types of malignant tumors at the same time by comprehensively statistically analyzing the methylation status of about 450,000 of the cytosines on DNA.

The methylation panel could identify the cancer cell of origin using the methylation profiles of several sets of clinical tissue samples, including primary, metastatic, and multiregional cancer samples, independent of the methylation analysis platform, specimen preparation method, ethnicity and geographic background. The methylation panel also could be applied to methylation data of circulating tumor DNA (ctDNA) samples from patients with malignant

tumor. Furthermore, the methylation panel is useful in identifying the tissue origin of cancer of unknown primary.

The results of this study are expected to develop into a screening technique that can accurately identify the location of malignant tumors using liquid biopsy such as blood sampling and urinalysis. In addition, the methylation panel could be used for accurate diagnosis of the primary lesion of cancer of unknown primary, suggesting that it may be possible to provide more accurate treatment for cancer of unknown primary.

This work was published online in *Cancer Gene Therapy* on November 8, 2021.

Research Background

The most common cause of death in Japan is malignant tumors, which is about 380,000, and is one of the serious social problems. Although the prognosis of patients with malignant tumors has improved due to advances in diagnostic equipment and the development of treatment methods, advanced malignant tumors have a high recurrence rate even after curative treatment, and it is generally difficult to curate after metastasis recurrence. The first opportunity to make a significant contribution as a medical intervention to reduce malignant tumor mortality is secondary prevention, namely early therapeutic intervention with early detection. Currently, there are only five organized cancer examinations (gastric cancer, colorectal cancer, lung cancer, breast cancer and cervical cancer) that are performed based on evidence on mortality reduction, and these examinations undergo separate examinations. The time, financial, and mental burdens should be one of the causes of the low examination consultation rate. In order to solve these problems, it is indispensable to develop a diagnostic method for malignant tumors with high accuracy ability and more versatility.

In recent years, ctDNA has been attracting attention as a target for testing malignant tumors. The existence of a malignant tumor can be proved if the somatic mutations specific for malignant tumors can be detected by blood test. However, these somatic mutations are common to various types of malignant tumor, so it is difficult to diagnose the location of a malignant tumor by testing somatic mutations. DNA methylation is one of the important processes of malignant tumor development and has organ specificity and tumor specificity. We conceived that DNA methylation is more useful for tumor site diagnosis than somatic mutations.

Research Results

We extracted cytosines that is specifically hypermethylated in each of the 27 types of malignant tumors by analyzing DNA methylation data of about 450,000 cytosines obtained from a total of 7950 cases of 28 types of malignant tumors registered in the public database. Next, by comparing with normal tissue DNA methylation data of 707 cases to focus on abnormal DNA methylation characteristical in malignant tumors, and comparing with whole blood DNA methylation data of 95 healthy subjects for application to blood samples, we generated a methylation panel including 2,572 cytosines (Figure 1A). The methylation panel was able to clearly cluster and distinguish each malignant tumor (Figure 1B). The set

threshold was able to diagnose 27 types of malignant tumors with high sensitivity and specificity (Figure 1C).

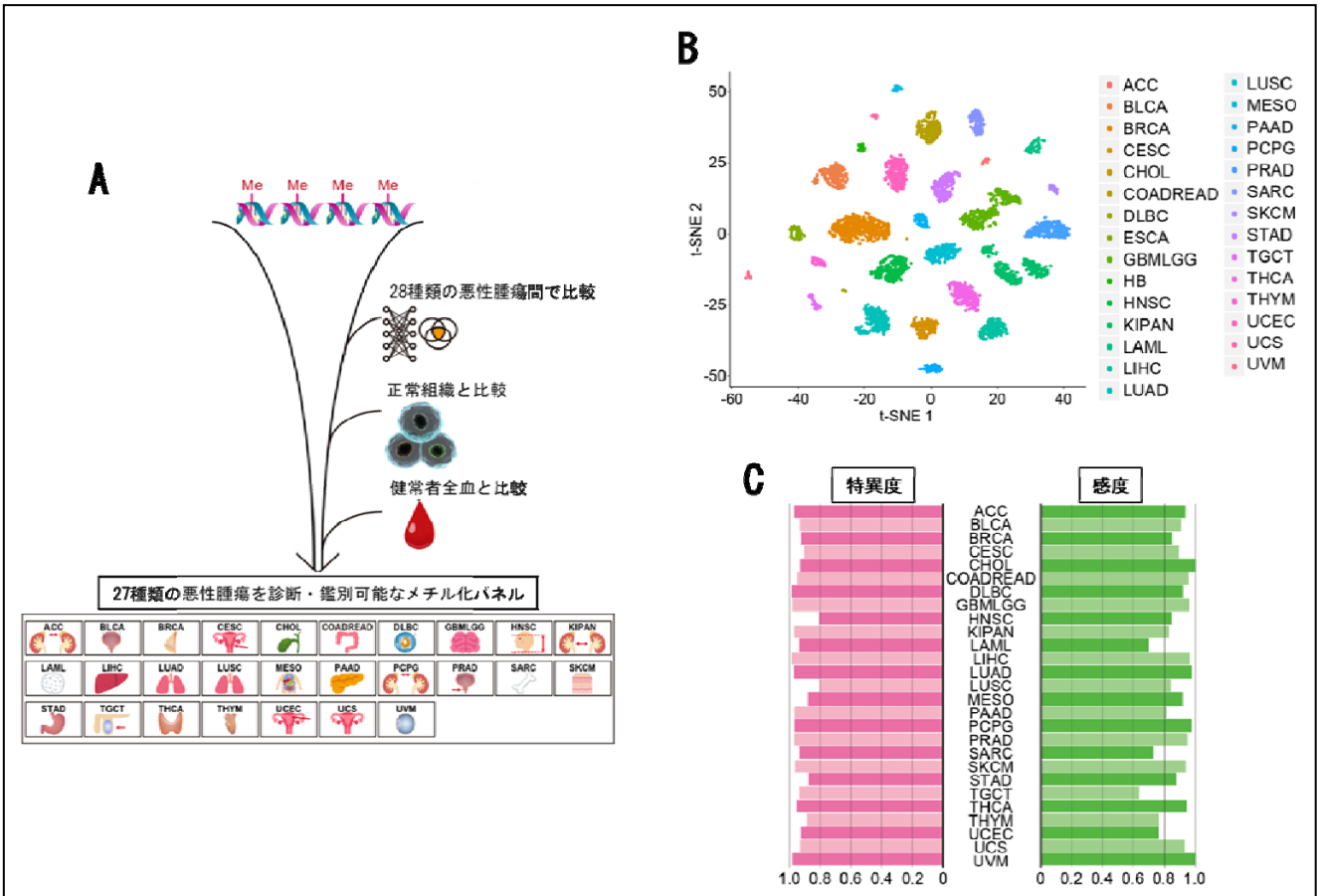

Figure 1. Generation of the methylation panel

A: By comprehensive statistical analysis of methylation data of 28 types of malignant tumors, normal tissues, and whole blood of healthy subjects, we created a methylation panel capable of diagnosing and distinguishing 27 types of malignant tumors. **B**: Clustering of 28 types of malignant tumor cases using the methylation panel (t-SNE analysis). **C**: Sensitivity and specificity of the methylation panel.

The diagnostic ability of the methylation panel was verified using the samples of malignant tumor tissue obtained at our institutions. Almost all frozen tissue samples of breast cancer (BRCA), colorectal cancer (COADREAD) and gastric cancer (STAD) analyzed by methylation array could be diagnosed as the appropriate malignant tumor (Figure 2A). The public data used to create the methylation panel is mainly composed of Western data. Since the samples used for verification were Japanese sample, it was suggested that the methylation panel could be applied regardless of ethnicity. Next, we verified diagnostic performance of the methylation panel using frozen primary sample and formalin-fixed paraffin-embedded metastatic samples of COADREAD analyzed by sequencing. Most samples could be diagnosed as colorectal cancer, and it was shown that the methylation panel could diagnose primary organ using metastatic sample and could applied independent of the methylation analysis platform and specimen preparation method (Figure 2B). We also verified diagnostic performance of the methylation

panel using multiregional COADREAD samples. Almost all tumor samples, both of primary and metastatic samples, were diagnosed as COADREAD and normal colorectal samples were completely distinguished from tumor tissue. Moreover, samples of the same case were clustered together. (Figure 2C). This result suggests that the diagnostic performance is not greatly affected by intratumor heterogeneity.

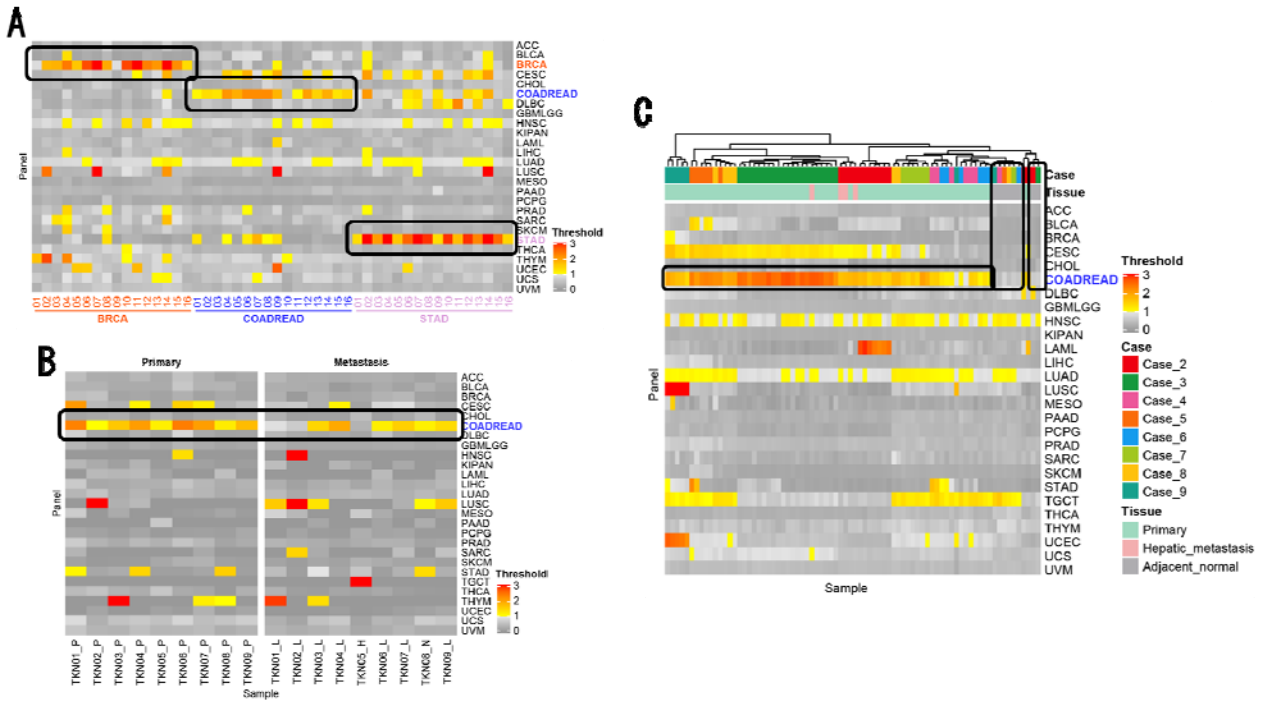

Figure 2. Verification of diagnostic performance using samples in our institutions

A: Frozen tissue samples of breast cancer (BRCA), colorectal cancer (COADREAD) and gastric cancer (STAD) were analyzed by methylation array. **B**: Frozen primary sample and formalin-fixed paraffin-embedded metastatic samples of COADREAD analyzed by sequencing. **C**: Multiregional COADREAD samples were analyzed by methylation array and cluster analysis.

We verified whether the methylation panel could be applied to blood sampling tests targeting ctDNA using methylation data of blood samples obtained from a public database. Blood sample data from health volunteer, BRCA, COADREAD, lung cancer (LUAD/LUSC) and cancer of unknown primary patients were used. The false positive rate in healthy samples was only 3.7%, and almost all samples of BRCA and COADREAD could be diagnosed as the appropriate malignant tumor (Figure 3A). On the other hand, it is difficult to detect lung cancer due to the small equivalent of ctDNA in blood samples. Future development of highly sensitive ctDNA methylation analysis technology is expected. In cancer of unknown primary cases, estimated primary lesion by the methylation panel was generally consistent with the primary lesion presumed from the clinical history (Figure 3B). These results suggested that the methylation panel could be a powerful tool for the noninvasive identification of cells of origin using liquid biopsy.

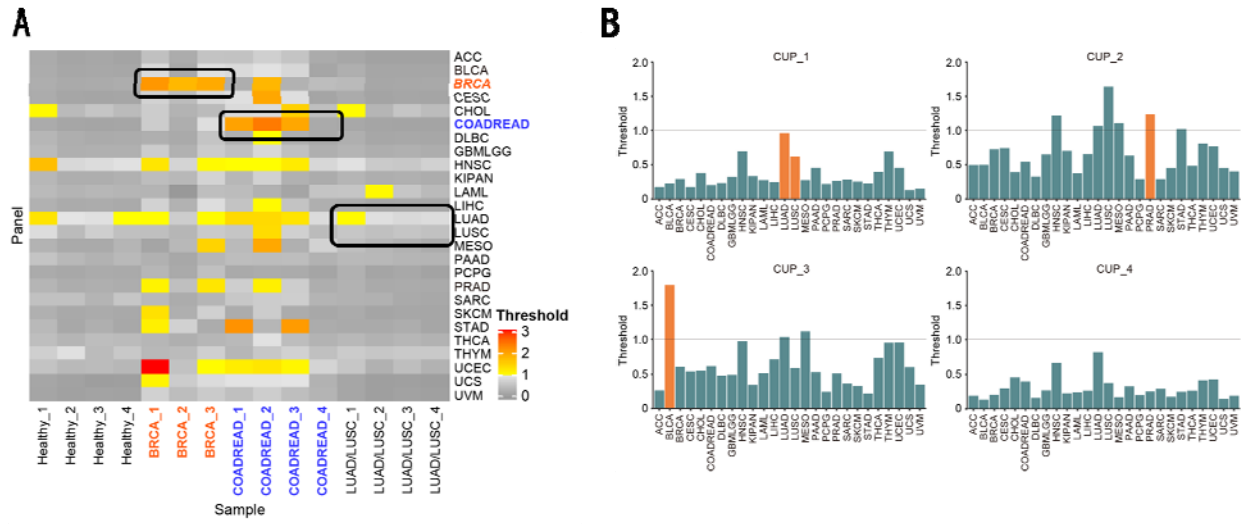

Figure 3. Verification of diagnostic performance using blood samples in public database

A: Blood sample data from health volunteer, BRCA, COADREAD, lung cancer (LUAD/LUSC) were applied to the methylation panel. **B**: Primary tumors were estimated using blood sample of cancer of unknown primary by methylation panel. Orange color indicates the presumed primary lesion from the clinical history.

Research Summary and Future Perspective

In this study, we developed a DNA methylation panel and showed that 27 types of malignant tumors can be diagnosed and distinguished mainly by using tumor tissue data. As the development of ctDNA methylation analysis technology advances, it will be possible to apply it to diagnosis by blood tests with higher accuracy. We aim to develop a screening technology that can diagnose up to 27 types of malignant tumors with a single blood sampling. Recently, it has been reported that urine also contains ctDNA. If our methylation panel can be applied to urinalysis, malignant tumor screening technology that can be received only by mailing urine will become possible.

Cancer of unknown primary is a metastatic malignant tumor whose primary lesion cannot be identified. Treatment of cancer of unknown primary is performed by estimating the primary organ from circumstantial evidence such as metastasis pattern, tumor marker and immunohistochemical staining. Since we showed that the primary organ can be diagnosed from the metastatic sample, the methylation panel should be useful for estimating the primary lesion of cancer of unknown primary. If the primary lesion can be diagnosed with higher accuracy based on the objective data of DNA methylation, more appropriate and effective treatment can be provided, and the prognosis of patients with cancer of unknown primary can be expected to improve.

Publication

Pan-cancer Methylome Analysis for Cancer Diagnosis and Classification of Cancer Cell of

Origin

Dai Shimizu, Kenzui Taniue, Yusuke Matsui, Hiroshi Haeno, Hiromitsu Araki, Fumihito Miura, Mitsuko Fukunaga, Kenji Shiraishi, Yuji Miyamoto, Seiichi Tsukamoto, Aya Komine, Yuta Kobayashi, Akihiro Kitagawa, Yukihiro Yoshikawa, Kuniaki Sato, Tomoko Saito, Shuhei Ito, Takaaki Masuda, Atsushi Niida, Makoto Suzuki, Hideo Baba, Takashi Ito, Nobuyoshi Akimitsu, Yasuhiro Kodera, Koshi Mimori

Cancer Gene Therapy, , published online on November 8, 2021.
DOI : 10.1038/s41417-021-00401-w

Japanese ver.

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