Progress report 2024

Next-Generation Precision Medicine Development Laboratory

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■ Overview

Cancer genomic medicine involves performing a comprehensive genomic profiling (CGP) test to identify gene mutations that can lead to targeted therapy. Since 2016, we have developed the Todai OncoPanel (TOP) at The University of Tokyo Hospital. Unlike other approved CGP tests that only have a DNA panel, TOP has a dual DNA/RNA panel. Furthermore, many polymorphic probes enable sophisticated gene copy number analyses. In April 2021, the Department of Next-Generation Precision Medicine Development Laboratory started as a social cooperation program, collaborating with Konica Minolta, Inc.

■ Our research

In our department, we are working on (1) validating the clinical utility of the TOP system and enhancing its multifunctionality, (2) optimizing pathological specimens for CGP tests, and (3).

(1) Clinical utility of Todai OncoPanel and further development

Background:

In August 2023, the TOP system was approved for reimbursement under the name "GenMineTOP Cancer Genomic Profiling System." This study aimed to evaluate the clinical utility of GenMineTOP in real-world practice under the national health insurance scheme.

Methods:

We analyzed GenMineTOP test results from 1,356 cases registered in the database of the Center for Cancer Genomics and Advanced Therapeutics (C-CAT). The DNA panel evaluated 737 genes, while the RNA panel assessed gene fusions involving 455 genes, exon skipping in 5 genes, and expression levels of 27 genes. Regarding *NTRK* gene fusions, we performed 10,000 simulations using FoundationOne CDx (F1CDx) data obtained from C-CAT, matching cancer types and patient ages with those in the GenMineTOP dataset. We also conducted multivariate analyses to investigate associations between RNA expression levels and copy number alterations or cancer types.

Results:

Oncogenic genomic alterations were identified in 91.5% of cases, including gene fusions in 105 cases. *NTRK* fusions were detected in 11 cases (0.8%) with GenMineTOP, showing a higher detection rate than F1CDx (median 0.44%, 95% CI: 0.147–0.739%) based on simulation. Additionally, diagnostically relevant gene fusions were detected in 49 cases. Correlation analysis between gene amplification and RNA expression revealed strong correlations for *MDM2*, *CDK4*, *EGFR*, and *ERBB2*, while genes such as *MYC* and *FGFR1* showed weaker correlations, indicating the need for caution in interpreting gene amplifications. Cancer type was the most influential factor affecting gene expression levels. Furthermore, certain mutations (e.g., *KIT* and *TERT*) and gene fusions (e.g., *ALK* and *RET*) were also associated with increased gene expression.

Discussion and Conclusion:

The RNA panel of GenMineTOP successfully

detected a variety of gene fusions. Notably, the higher detection rate of *NTRK* fusions compared to F1CDx may be partially attributed to the absence of *NTRK3* intron probes in F1CDx; while *ETV6-NTRK3* fusions can be detected via the *ETV6* probe, fusions involving other partners may be missed. The RNA expression data obtained may serve as a valuable resource for interpreting future test results.

(2) Best tissue processing practices for CGP tests with RNA panel

Background:

In CGP testing, unstained formalin-fixed paraffin-embedded (FFPE) specimens used for pathological diagnosis are widely utilized. The processes from specimen collection to pre-analytical fixation significantly affect nucleic acid quality and sequencing success, highlighting the need for optimization. This study aimed to investigate the influence of specimen size, time to fixation (cold ischemia time), fixative concentration, and fixation duration on DNA and RNA yield and quality.

Methods:

Fresh porcine liver tissue obtained within 30 min post-excision was used to prepare specimens of three sizes. Some experiments have also included porcine pancreas.

Specimen1: Simulating biopsy specimens (2 mm thick, 2 mm × 15 mm)

Specimen2: Ideal specimen size
(10 mm thick, 12 mm × 10 mm)

Specimen3: Simulating “real-world” surgical specimens
(50 mm thick, 300 mm × 50 mm)

FFPE specimens were prepared under the following conditions:

- Time to fixation (30 min, 3 h, 6 h, 24 h)
- Fixative concentration (10% and 20% neutral buffered formalin [NBF])
- Duration of fixation (1, 3, and 7 days).

Specimens 1 and 2 were cut into uniform sizes using a biopsy punch and embedded in three pieces per block (n=3). Specimen 3 was fixed without appropriate pre-trimming and subsequently cut into 5 mm cubes before embedding (three pieces per block, n=3). DNA and RNA were extracted from a single 10 μm section and evaluated for yield and quality using a Qubit fluorometer and TapeStation.

Results:

1. Effect of Specimen Size

in large specimens (50 mm-thick), DNA quality and yield decreased, while RNA quality and yield remained unaffected.

2. Effect of Time to Fixation

In the liver tissue stored in moist conditions, no significant changes were observed, even with a 24-hour delay before fixation. In contrast, the pancreatic tissue showed a degradation in DNA quality after 3 h.

3. Effect of Fixative Concentration

Compared to 10% NBF, 20% NBF resulted in reduced DNA quality, whereas RNA quality remained unaffected.

4. Effect of Fixation Duration

Regardless of specimen size, fixation for more than three days led to decreased DNA and RNA quality.

Discussion and Conclusion:

This study revealed that pre-fixation and fixation conditions affect the quality of DNA and RNA differently. To obtain high-quality DNA and RNA suitable for CGP testing, it is desirable to initiate fixation in 10% neutral buffered formalin (NBF) within 3 h post-excision, to apply appropriate trimming for large specimens prior to fixation, and to limit the fixation duration to 1 d (24 h). These conditions contribute to the standardization of FFPE specimen preparation and ensure a reliable nucleic acid quality for molecular diagnostics.

(3) Evaluation of the Clinical Utility of Tumor-Derived Circulating HPVDNA in Early-Stage Cervical Cancer

Background:
More than 95% of cervical cancer cases are caused by HPV infection, with HPV types 16 and 18 being the most frequently detected. In recent years, “liquid biopsy,” a non-invasive technique for measuring biomarkers in blood and other body fluids, has attracted significant attention. Tumor-derived circulating HPVDNA (ctHPVDNA) is expected to serve as a novel biomarker. This study aimed to evaluate the detectability of ctHPVDNA and its clinical relevance in early-stage cervical cancer.

Methods:
Among 109 patients scheduled for surgery for early-stage cervical cancer, 24 cases for which both tumor tissue and preoperative peripheral blood samples were available were included in the study. The measurement of ctHPVDNA was performed using highly sensitive and quantitative digital PCR (dPCR). Type-specific assays targeting high-risk HPV types (16, 18, 31, and 52) were designed.

Results:
Among 22 cases in which HPV genotypes were identified from tumor DNA, the detection rate of ctHPVDNA was 31.8% (7/22 cases). Although ctHPVDNA levels did not significantly correlate with tumor size, clinical stage, or recurrence rate, cases with lymphovascular invasion tended to have higher ctHPVDNA levels, and ctHPVDNA-positive cases showed a higher rate of lymphovascular invasion ($p = 0.069$). Similarly, cases with lymph node metastasis tended to have higher ctHPVDNA levels. Among ctHPVDNA-negative cases, the negative predictive value for lymph node metastasis was 73%.

Discussion and Conclusion:
These findings suggest that ctHPVDNA may reflect the extent of lymphovascular invasion rather than local tumor burden. ctHPVDNA could serve as a useful adjunctive indicator for preoperative assessment of disease progression in early-stage cervical cancer.

■ Future directions

(1) Clinical utility of Todai OncoPanel and further development

Our department is also conducting the TOP2 prospective study to enhance the multifunctionality of the TOP system beyond its use within the framework of insurance-covered clinical practice. This includes the development of a homologous recombination deficiency (HRD) scoring system in the DNA panel and the evaluation of expression signatures in the RNA panel—such as primary site prediction, sensitivity to immune checkpoint inhibitors, and prognosis estimation—aimed at expanding the functional capabilities of TOP.

(2) Best tissue processing practices for CGP tests with RNA panel

Future studies will extend these investigations to a broader range of organs and tumor tissues to determine the optimal fixation conditions for each tissue type. Validation using clinical specimens will be conducted to assess the applicability of these findings in the diagnostic and therapeutic settings. Additionally, automation and standardization of fixation protocols will be pursued to improve reproducibility across institutions.

(3) Evaluation of the Clinical Utility of Tumor-Derived Circulating HPVDNA in Early-Stage Cervical Cancer

We aim to promote the clinical application of ctHPVDNA by increasing the number of cases for further validation and conducting molecular genetic analyses using cancer gene panels.

■ Research activities for fiscal year 2024

Publications:

1. Isago H, Watanabe K, Satoh Y, Kurano M. Correlation between variant call accuracy and quality parameters in comprehensive cancer

genomic profiling tests.
Pract Lab Med 2024;39:e00369. doi:10.1016/j.plabm.2024.e00369. PMID:38404524

2. Watanabe K, Kohsaka S, Tatsuno K, Shinozaki-Ushiku A, Isago H, Kage H, Ushiku T, Aburatani H, Mano H, Oda K.
Analysis of quality metrics in comprehensive cancer genomic profiling using a dual DNA-RNA panel.
Pract Lab Med 2024;39:e00368. doi:10.1016/j.plabm.2024.e00368. PMID:38404525

3. Ando T, Ka M, Sugiura Y, Tokunaga M, Nakagawa N, Iida T, Matsumoto Y, Watanabe K, Kawakami M, Sato M, Kage H.
NECTIN2 is a prognostic biomarker and potential therapeutic target in lung adenocarcinoma.
Respir Investig 2024;62(4):582–588. doi:10.1016/j.resinv.2024.04.002. PMID:38678829

4. Kage H, Kohsaka S, Tatsuno K, Watanabe K, Shinozaki-Ushiku A, Isago H, Ushiku T, Aburatani H, Mano H, Oda K.
Molecular analysis of non-small cell lung cancer using a dual-targeted DNA and RNA comprehensive genomic profiling panel.
Respir Investig 2024;62(5):910–913. doi:10.1016/j.resinv.2024.07.018. PMID:39126824

5. Ka M, Matsumoto Y, Ando T, Hinata M, Xi Q, Sugiura Y, Iida T, Nakagawa N, Tokunaga M, Watanabe K, Kawakami M, Ushiku T, Sato M, Oda K, Kage H.
Integrin-α5 expression and its role in non-small cell lung cancer progression.
Cancer Sci 2025;116(2):406–419. doi:10.1111/cas.16416. PMID:39581761

6. Matsushita K, Ishige T, Watanabe K, Akahane T, Tanimoto A, Yoshimoto M, Yamakuchi M, Hashiguchi T, Okugawa Y, Ikejiri M, Yamaguchi T, Yamasaki T, Takeda M, Hibi M, Akiyama N, Shimizu K, Hashimoto N, Sato H, Tanaka Y, Amari F; EQA working group of Japan Association for Clinical Laboratory Science (JACLS).
Importance of EQA/PT for the detection of genetic variants in comprehensive cancer genome testing.
Sci Rep 2025;15(1):1036. doi:10.1038/s41598-024-84714-4. PMID:39762492

7. Fujii K, Ueki M, Morishita M, Ikushima H, Isago H, Watanabe K, Oda K, Kage H.
Clinical utility of comprehensive genomic profiling in non-small cell lung cancer: An analysis of a nation-wide database.
Lung Cancer 2025;200:108099. doi:10.1016/j.lungcan.2025.108099. PMID:39842065

8. Xi Q, Kunita A, Ogawa M, Ka M, Tanimoto S, Tsuchimochi S, Nagai S, Matsunaga A, Fukuda T, Watanabe K, Sone K, Shinozaki-Ushiku A, Kawana K, Ushiku T, Osuga Y, Katayama K, Kage H, Oda K.
Cyclin E1 overexpression sensitizes ovarian cancer cells to WEE1 and PLK1 inhibition.
Oncogene 2025 Feb 24. doi:10.1038/s41388-025-03312-4. Epub ahead of print. PMID:39994376

9. Ikushima H, Watanabe K, Shinozaki-Ushiku A, Oda K, Kage H.
A machine learning-based analysis of nationwide cancer comprehensive genomic profiling data across cancer types to identify features associated with recommendation of genome-matched therapy.
ESMO Open 2024;9(12):103998. doi:10.1016/j.esmoop.2024.103998. PMID:39591805

10. Ikushima H, Watanabe K, Shinozaki-Ushiku A, Oda K, Kage H.
Pan-cancer clinical and molecular landscape of MTAP deletion in nationwide and

international comprehensive genomic data.
ESMO Open In press.

11. Watanabe K, Ogawa M, Shinozaki-Ushiku A, Tsutsumi S, Tatsuno K, Aburatani H, Kage H, Oda K.
Real-world data analysis of genomic alterations detected by a dual DNA-RNA comprehensive genomic profiling test.
Cancer Sci. 2025 Epub ahead of print. doi: 10.1111/cas.70071. PMID: 40140952.

12. Rokutan H, Arai Y, Kunita A, Yamasaki S, Nakamura H, Hama N, Nakayama A, Hosoda F, Totoki Y, Fujishiro M, Seto Y, Shibata T, Ushiku T.
Genomic and Pathologic Profiling of Very Well-Differentiated Gastric Adenocarcinoma of Intestinal Type: A Study With Emphasis on Diffuse-Type Transformation. Am J Surg Pathol 2024, 48:652-61. doi:10.1097/PAS.0000000000002222.
PMID: 38584451

13. Tanaka M, Takeshita K, Kunita A, Hasegawa K, Ushiku T.
PRKACA/PRKACB Fusions in Pancreatobiliary Intraductal Oncocytic Papillary Neoplasms Including Those With Atypical Morphology: An Analysis of 22 Cases Expanding Morphologic Spectrum. Am J Surg Pathol 2024, 48:1032-40. doi:10.1097/PAS.0000000000002259.
PMID: 38841868

International conferences:

1. Ueki M, Watanabe K, Morishita M, Fujii K, Ikushima H, Isago H, Oda K, Kage H.
Incidence of pathogenic germline variants and presumed germline pathogenic variants in Japanese lung cancer patients using comprehensive genomic profiling tests.
American Association for Cancer Research Annual Meeting 2024, Poster Presentation.

2. Morishita M, Fujii K, Ueki M, Ikushima H, Isago H, Watanabe K, Oda K, Kage H.
Real-world data analysis of comprehensive genomic profiling using plasma samples from non-small cell lung cancer patients.
American Association for Cancer Research Annual Meeting 2024, Poster Presentation.

3. Fujii K, Morishita M, Ueki M, Ikushima H, Isago H, Watanabe K, Oda K, Kage H.
The real-world data of comprehensive genomic profiling tests with tissue specimens from lung cancer patients in Japan.
American Association for Cancer Research Annual Meeting 2024, Poster Presentation.

4. Tokunaga M, Nakagawa N, Ka M, Sugiura Y, Iida T, Ando T, Watanabe K, Kage H, Kawakami M.
Screening of drug library targeting neural signaling identifies cholecystokinin B receptor as a potential therapeutic target in small cell lung cancer.
American Association for Cancer Research Annual Meeting 2024, Poster Presentation.

5. Kage H, Aoki T, Shinozaki-Ushiku A, Watanabe K, Akiyama N, Isago H, Ogawa M, Ishigaki K, Kobayashi H, Miyagawa T, Omatsu J, Saito Y, Sasaki K, Sato Y, Sato Y, Suzuki N, Tanaka S, Kato M, Tanabe M, Tatsuno K, Ushiku T, Nishimura K, Aburatani H, Oda K.
Use of artificial intelligence to facilitate reporting of comprehensive genomic profiling tests.
American Association for Cancer Research Annual Meeting 2024, Poster Presentation.

6. Nakagawa N, Tokunaga M, Ka M, Sugiura Y, Iida T, Ando T, Watanabe K, Liu X, Dmitrovsky E, Kage H, Kawakami M.
KIFC1 inhibition elicits antineoplastic activity in small cell lung cancer by inducing anaphase catastrophe.
American Association for Cancer Research Annual Meeting 2024, Poster Presentation.

7. Losic B, Tatsuno K, Singan VR, Ueda H, Shinozaki-Ushiku A, Jayakumar V, Hattori E, Ichijo T, Sasa T, Akahori M, Hayashi S, Hsiao E, Feupe S, Vo D, Geier T, Tsutsumi S, Ushiku T, Watanabe K, Oda K, Kage H, Radecki-Crandall J, Cheng DT, Aburatani H.
Integrated multi-omic/modal profiling enables improved characterization of oncogenic processes, with impact to clinical actionability in the GenMineTOP tested population.
American Association for Cancer Research Annual Meeting 2024, Poster Presentation.

8. Ikushima H, Watanabe K, Shinozaki-Ushiku A, Oda K, Kage H.
A retrospective machine learning-based analysis of nationwide cancer comprehensive genomic profiling data to identify features associated with recommendation of mutation-based therapy.
American Society of Clinical Oncology Annual Meeting 2024, Poster Presentation.

9. Ikushima H, Watanabe K, Shinozaki-Ushiku A, Oda K, Kage H.
A retrospective machine learning-based analysis of nationwide cancer comprehensive genomic profiling data to identify features associated with recommendation of mutation-based therapy.
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