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GC-MS GCMS-TQ™ 8040 NX

Application News

Facilitation of Liquid Biopsy Research using a GC-MS and a Circulating Tumor Cells Isolator

Yuki Nakagawa[1], Hirokazu Moriya[2]
1 Shimadzu Corporation, 2 CytoGen, Inc.

User Benefits

- Because GC-MS detect trace amounts of metabolites (e.g., sugars, amino acids, fatty acids, nucleic acids, organic acids, etc.), it facilitates studies of tumor metabolism by culturing living tumor cells and measuring their culture media.
- By using Multi-omics Analysis Packages, not only primary metabolite measurement data, but also cell and culture media data can be read to detect measurement items (digital biomarkers) with characteristic features.

■ Introduction

In recent years, liquid biopsy has attracted attention as a breakthrough technology in cancer research. Liquid biopsy, which uses samples such as urine and blood to detect cancer at an early stage and monitor the effects of treatment, is said to be less invasive and easier to repeat than conventional tissue biopsies.

In the development of early detection and treatment of cancer using liquid biopsy, ctDNA is usually the focus. However, since only DNA can be measured with ctDNA as the name implies, an analysis of circulating tumor cells (CTCs) such as RNA and protein is becoming increasingly important (Fig. 1).

Isolating living CTCs from blood, concentrating them, and understanding the changes in trace amounts of metabolites contained in the culture medium in which the cancer cells are cultured could make a significant contribution to liquid biopsy research. GC-MS (gas chromatography mass spectrometry) measures primary metabolites in culture media.

However, there are challenges with GC-MS in terms of expertise and metabolic pathway development. To overcome these challenges, this application used GCMS™-TQ8040 NX (Fig. 2 left) together with Smart Metabolites Database™ Ver. 2.

Using a database eliminates the need for specialized knowledge and technology required for method development. The simultaneous measurement of more than 500 components was completed in 23 minutes. The samples used were 2 types of CTCs, H358 and H1975 lung cancer cells, separated by Cytogen's Smart Biopsy Cell Isolator (Fig.2 right) and cultured in individual culture media with 0, 1, 10, and 100 cells, respectively. The Smart Biopsy Cell Isolator uses gravity-based filtering with HDM to maximize the recovery rate with a special coating to isolate live CTCs in a high-throughput manner in 25 minutes per sample. Statistical analysis was performed using eMSTAT™ Solution Ver. 2 and Multi-omics Analysis Package, using a visualization method combining ANOVA and a metabolic pathway diagram, as well as visualizing and analyzing correlations between CTC count data and metabolites on the metabolic pathways.

Fig. 2 GCMS-TQ™8040 NX (left) and Smart Biopsy Cell Isolator (right)

Good potential in the future **miRNA**	miRNAs affect the circulating cells and are suitable for early detection of cancer. Its DNA, RNA, and even proteins to some extent, can be analyzed. However, in addition to high-sensitivity analysis tools, correction of miRNA abundance is required. Cancer-specific gene information analysis is likely not feasible. Although miRNAs are enclosed in exosomes or are free in the blood, they are stable in exosomes and may be used as markers.
becoming popular now **CTC** (Circulating Tumor Cells)	Tumor cells (i.e., rare cells as in one of hundreds of millions of cells) require "single-cell isolation technology" and are difficult to isolate. However, it can evaluate DNA, RNA, proteins, organelles (3D imaging), and cell properties (hardness, etc.) just like the tumor tissue itself. When detected, it is reported that the agreement rate with analytical results, such as ctDNA, is high and the accuracy is significant.
Most frequently studied **ctDNA** (circulating tumor DNA)	The current mainstream of liquid biopsy research is ctDNA. Mononuclear cells are abundant and easily separated from peripheral blood. The disadvantage is that only DNA can be analyzed, and RNA, proteins, organelles, and cell properties cannot be observed. Problems include contamination with external DNA such as DNA derived from normal cells, and the lack of established evaluation methods.
EV (Extracellular vesicle)	They are vesicles that are released from cells and do not have a nucleus and cannot replicate. They require careful handling because they are destroyed by freezing and thawing. It can analyze DNA, RNA, and protein, but there are currently few research cases compared to ctDNA.
cfDNA (circulating free DNA)	Since the origin of cfDNA is difficult to determine, the number of dead cells in the whole body (advanced cancer ➡ increase in the number of dead cells ➡ increase in the total amount of cfDNA (normal cells + cancer cells + inflammatory cells)) is probably observed. The number of dead cells may correlate with the degree of invasiveness of surgery and is also a promising marker of surgical damage.
cfRNA (circulating free RNA)	Analytical techniques are notoriously difficult. However, compared to cfDNA, it is more suitable for identification of fusion genes (genes involved in cancer development)

Fig. 1 Overview of Liquid Biopsy Research Targets

■ Experimental

Blood samples were collected in acidic citrate glucose A tubes (BD; Franklin Lakes, NJ, USA). Blood samples were processed using the CTC Isolation Kit (Part Number CIKW10; Cytogen Inc.) and SMART BIOPSY Cell Isolator (Part Number CIS030; Cytogen Inc.) to enrich for CTCs. The diluted cell suspension was filtered through a high-density microporous (HDM) tip. The cells on the HDM chip were collected and transferred to microtubes.

Human lung cancer cells H358 (wild-type EGFR) and H1975 (L858R and T790M) were incubated with 10 % heat-inactivated fetal bovine serum and 5 ml antibiotic-antimycotic (Product No. 15240062).; L-glutamine (300 mg/L) (Product No. 11875093) supplemented with; Gibco) in RPMI 1640 medium. They were cultured at 37 ˚C in a humidified 5 % CO2 atmosphere.

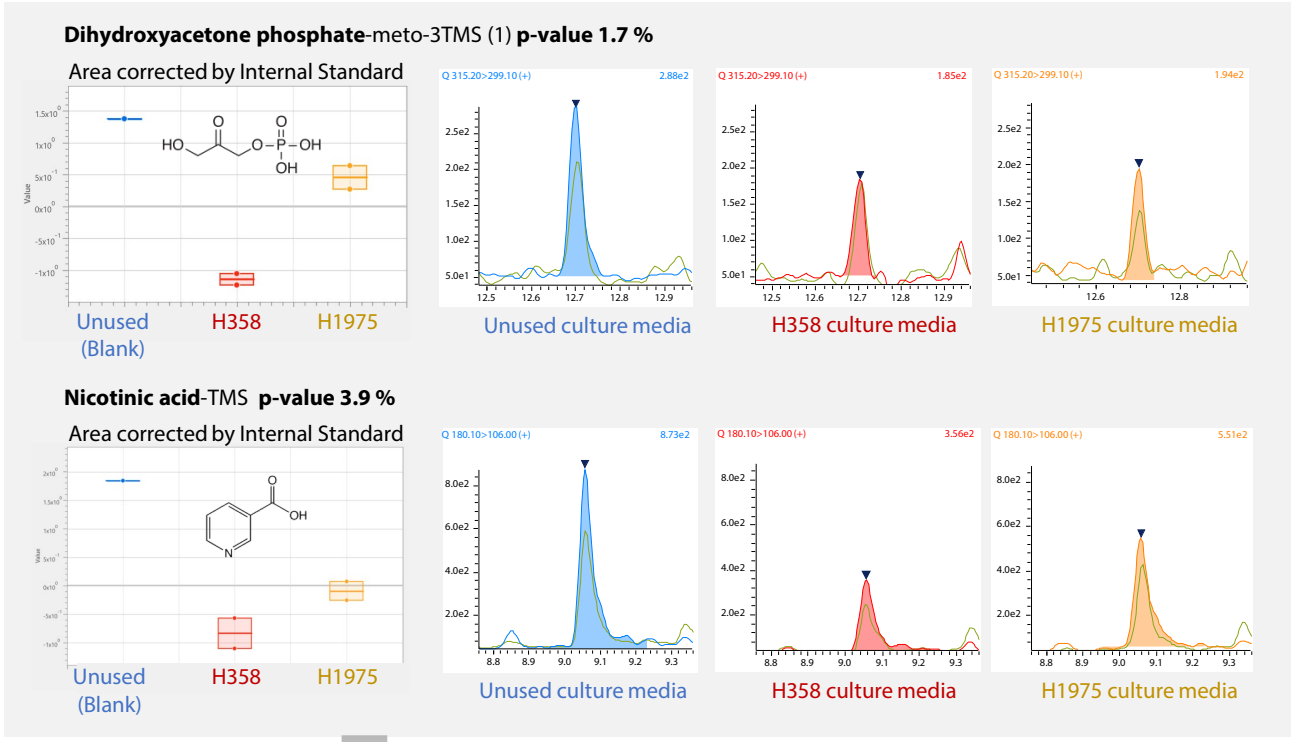
To determine the effect on the metabolite profile of isolated and cultured CTCs, 11 media samples (Unused medium, medium containing 1 cell for each lung cancer (n=2), medium containing 10 cells, medium containing 50 cells, medium containing 100 cells) were measured on a GCMS-TQ8040 NX.

Sample preparation was performed in accordance with the Metabolomics Pretreatment Handbook, and GC-MS analysis was performed using Smart Metabolites Database Ver. 2. Using Multiple Reaction Monitoring (MRM) as the data collection mode, more than 500 compounds were measured with high sensitivity within 23 minutes of analysis time.

■ Metabolic pathway analysis with ANOVA

ANOVA (i.e., ANalysis Of VAriance) is used to test for differences in data between groups and to determine whether the differences are due to chance. The results of ANOVA are usually only p-values, but by projecting the results onto metabolic pathways, biological knowledge are deepened and new directions for research may be found.

In Fig. 3, p-values were calculated by ANOVA for unused medium (blue), medium containing 50 or 100 lung cancer cells (H 358) (red), and medium containing 50 or 100 lung cancer cells (H 1975) (yellow). Metabolites with p-values of 5 % or less were projected onto the metabolic pathway map of Multi-omics Analysis Package.



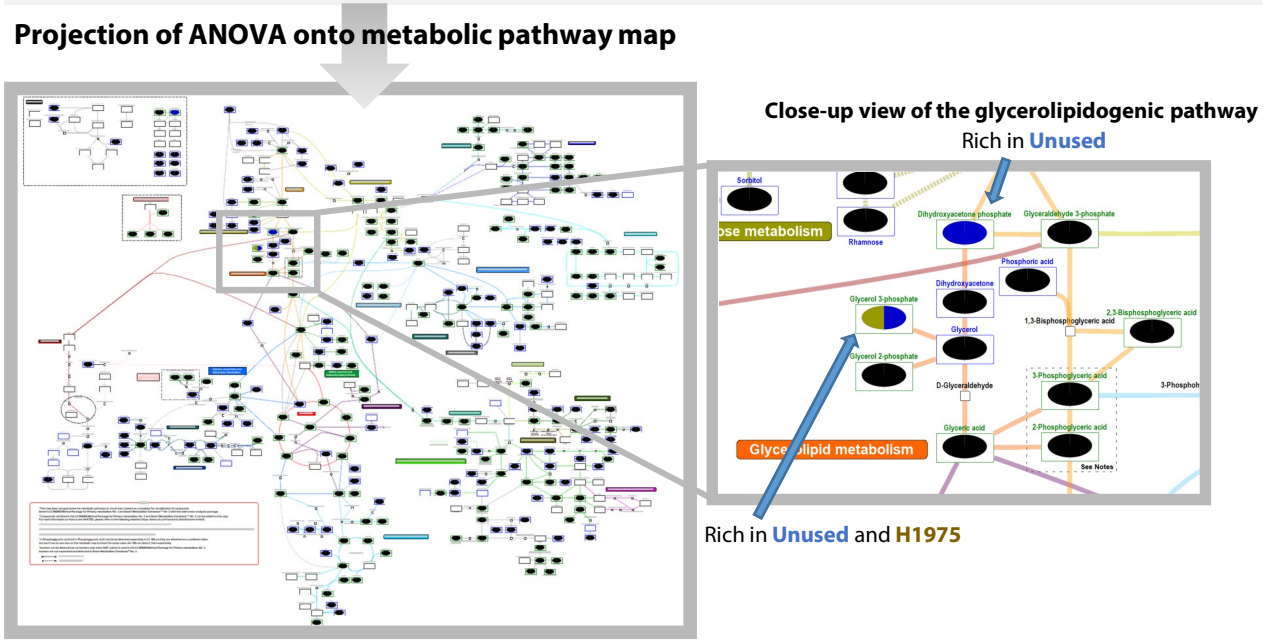


Fig. 3 ANOVA analysis performed with eMSTAT Solution Ver,2 and the results projected onto the metabolic pathway map of Multi-omics Analysis Package

As a result, the substrate (dihydroxyacetone phosphate and glycerol 3-phosphate) in the upstream region of the glycerolipid metabolic pathway was specifically low in the culture medium of H358. Methylglyoxal is a by-product from dihydroxyacetone phosphate, and cancer cells tend to produce Methylglyoxal as a by-product due to increased glycolysis[1].

■ Correlation Analysis of CTC Numbers and Metabolites on Metabolic Pathway

Using the metabolic pathway analysis function of Multi-omics Analysis Package, a correlation analysis between the number of CTCs (0, 1, 10, 50, 100) and metabolites was performed (Fig. 4).

The results showed that dopamine, an intermediate metabolite in the tryptophan pathway, had a positive correlation with H358. Plasma dopamine levels in lung cancer patients are known to be significantly higher than those in healthy subjects, and the causal relationship is expected to be elucidated in the future[2].

In the glycerolipidogenic pathway, glycerol 3 phosphate was found to be negatively correlated with H358 cell number. It is known that glyceraldehyde -3 phosphate dehydrogenase (NADP+), which converts glycerol 3 phosphate to 1, 3 bisphosphoglycerate, is highly expressed in lung cancer cells[3]. Therefore, the reason for the decrease in glycerol 3 phosphate in this experiment is also considered to be an increase in glyceraldehyde -3 phosphate dehydrogenase (NADP+).

■ Summary

In this study, H358 and H1975 lung cancer cells were isolated and cultured using Cytogen's Smart Biopsy Cell Isolator. Culture media were analyzed with GCMS-TQ8040 NX and Smart Metabolites Database Ver. 2, and metabolites correlated with CTC counts were detected using Multi-omics Analysis Package. We hope that isolating and culturing live CTCs using a cell-size filtration method and negative selection and measuring the culture medium by GC-MS will facilitate research on tumors.

<References>

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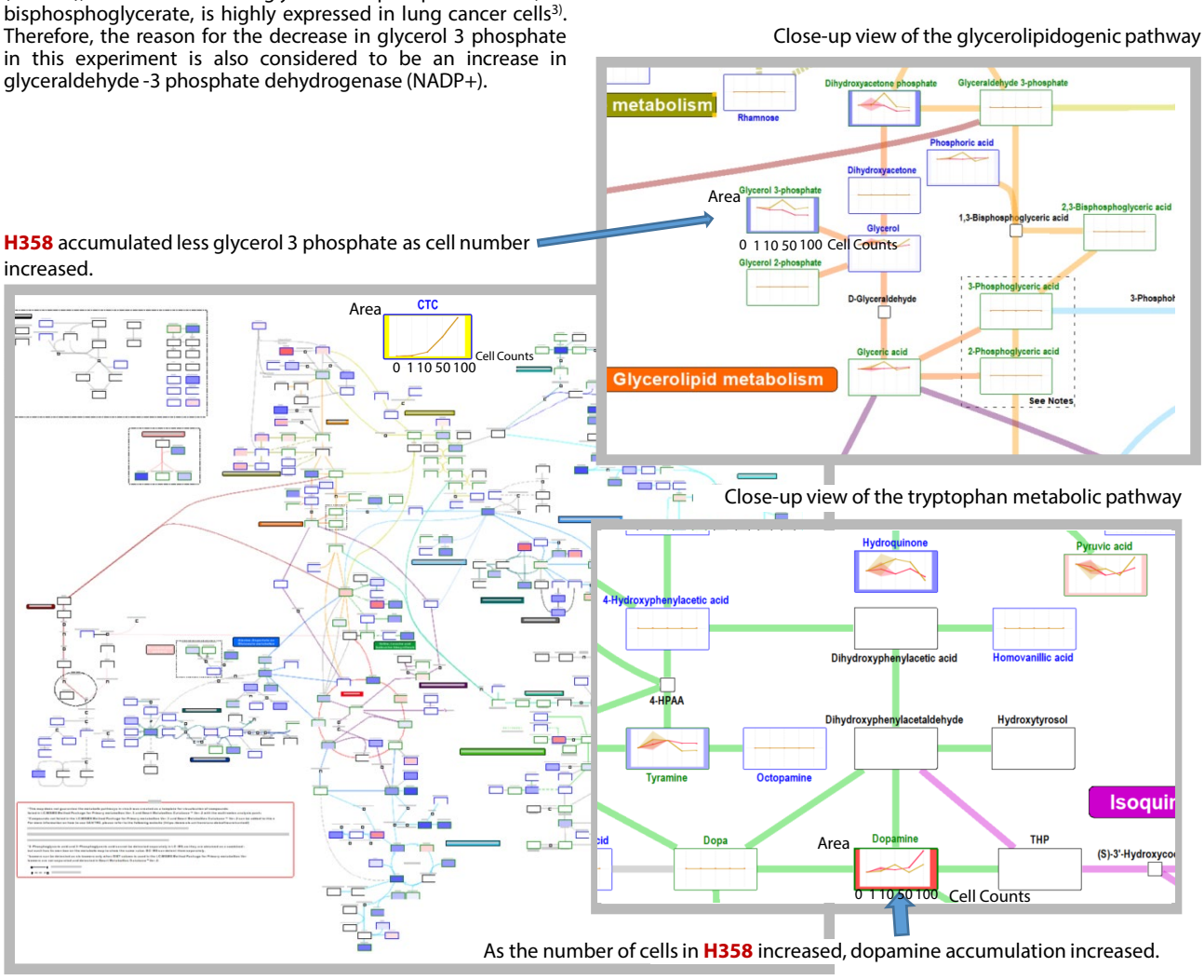

Fig. 4 Search for metabolites correlated with CTC counts

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