Cell³iMager

■ Discussion

The spheroid area measured by scanner in 3D culture was well correlated with ATP level during a limited time of culture. In addition, the compounds-induced reduction of spheroid area in the secondary screening showed high correlation with the decrease in ATP, validating the spheroid area as an index of spheroid growth.

The screening was done at relatively low compound concentration (up to 1000 nM maximum). However, the primary screening identified 39 compounds that reduced spheroid area in the 3D spheroid culture of 293T cells. Of these compounds, the molecularly-targeted drugs such as mTOR, PDGFR, Hsp90 inhibitors exhibited cancer-cell selective (293T-selective) inhibitory effects. Thus, use of the 3D spheroid culture to screen cancer cells can facilitate the selection of compounds that will most effectively inhibit cancer cell growth in the human body.

■ Conclusions

● We have developed a fast, easy-to-use, reagent-free, inexpensive, and high-throughput screening method for anticancer drugs using a high-speed cell scanner (Cell³iMager). This method measures and indexes the sizes of 3D cancer cell spheroids.

● To verify the usability and practicability of this screening method, we screened Standard Inhibitor Kits #1-4 (360 compounds comprising approved anticancer drugs and various molecular-targeted inhibitors) provided by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan and its supporting research institutions. The present study indicated that the some targeted drugs including the mTOR inhibitor exhibited a selective growth-inhibiting effect on cancer cells.

Courtesy：Nagahama Institute of Bio-Science and Technology Mizukami Laboratory

SCREEN

High-Speed 3D Cell Scanner Application Note

" Development of spheroid-based anticancer drug screening method using high-speed cell culture scanner "

Cell³iMager

A Bright Field Spheroid Counter,
Allowing Reagent-Free Follow-up,
Ideal for Label-Free Assays of 3D Cell Culture

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■ Purpose of the study

In recent years, three-dimensional (3D) cell culture has become increasingly valuable in the research and development of anticancer drugs. Compared with conventional two-dimensional (2D) cell culture, 3D culture is considered to more accurately approximate the environment of the body, thus enabling more accurate evaluation of drug efficacy. In addition, because many conventional drug susceptibility tests interrupt the culture process by adding a test reagent to measure the number of living cells, such tests are not only time-consuming but also do not allow for the observation of progress over the same cells.

In 2013, Dainippon Screen Mfg. Co., Ltd. introduced the High-Speed 3D Cell Scanner Cell³iMager scanner to address these issues. This scanner uses image-processing technology to measure and analyze the growth and morphological changes over time of cancer cells cultured on the 3D culture plate, thus eliminating the need for test reagents.

In this study we used the Cell³iMager to develop a fast, easy-to-use, inexpensive, and high-throughput screening method for anticancer drugs. Using two human cancer cell lines (HEK293 and HEK293T), here we show that the area of spheroid formed in 3D culture is well correlated with the growth of cells and that anticancer drugs can be rapidly screened.

■ Materials

●Cell lines

HEK293 (293) cells were generated by transformation of normal human embryonic kidney cells with sheared adenovirus 5 DNA. HEK293T (293T) cells were further transformed with SV40 virus large T-antigen. When we tested both cell lines in 3D culture, we found that 293 cells form tight, spherical and regular spheroids and 293T cells form similar spheroids with higher growth rate. Thus, we expected that both cell lines are useful to screen anticancer drugs with Cell³iMager. In this study, we used 293 cells as cancer cells with low malignancy and 293T cells as cancer cells with high malignancy.

●Compound library

Standard Inhibitor Kits #1-4 were provided by the Screening Committee of Anticancer Drugs (SCADS), supported by a Grant-in-Aid for Scientific Research on Innovative Areas, Scientific Support Programs for Cancer Research, from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan. These kits contain 360 compounds comprising a huge variety of molecular-targeted anticancer agents. The authors wish to express their deep gratitude for this support.

●High-Speed 3D Cell Scanner "Cell³iMager"

This system allows spheroid analysis by the scanning of well plates in a bright field. It calculates the number, area, and volume of the spheroids based on optical density, in the well plates without using reagents.

The pixel size can be set to 10 μm, 5 μm, or 2.5 μm.

If a measurement recipe has already been set, a plate can be scanned in one minute, and an automatic measurement can be completed within about 30 seconds after scanning.

[Product information]
http://www.screen.co.jp/pd/products/3d/cell3_imager/

■ Method

●Cell growth and spheroid formation

HEK293 and HEK293T cells for 3D culture were seeded at a density of 500 cells in 100μl per well on a PrimeSurface® 96U plate, a 96-well ultralow-cell-adhesion plate (Sumitomo Bakelite Co., Ltd., P/N MS-9096U) with a U-shaped bottom for producing single spheroids.

From Day 2 to Day 11 after seeding, spheroid growth was followed by measuring the areas of spheroids using the scanner, and by ATP quantitative assays using the living cell counting protocol (CellTiter-Glo®, Promega). All experiments were done in triplicate (n = 3).

●Primary screening

On Day 1 after seeding, spheroid areas were measured for 3D culture. The values measured at this point were set as the values for zero hours after the agents were added.

Compounds from the standard inhibitor kit were used at final concentrations of 10 nM, 100 nM, and 1000 nM. The vehicle (DMSO) was added as control.

Forty-eight hours after the compounds were added, spheroid areas were measured for 3D culture. The 50% inhibitory concentration (IC50) of each compound was calculated. Compounds that reduced spheroid areas of 293T cells in the 3D culture with a high selective manner were selected for secondary screening.
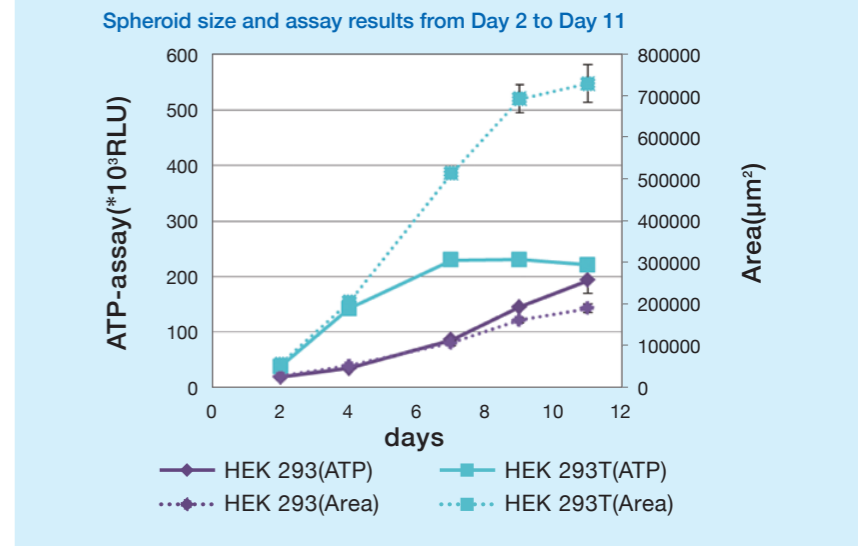
●Secondary screening

The secondary screening was performed with the same procedures used for the primary screening. In addition, ATP contents in spheroids in the 3D culture were assayed.

■ Results

●High correlation between spheroid areas and ATP values

Figure 1 shows areas of spheroids measured by the scanner and the ATP assay values. High correlation between the spheroid areas and ATP values was confirmed up to Day 4 after seeding. Therefore, to screen compounds that inhibit spheroid growth, they were added on Day 1, and the areas were measured on Day 3.

[Figure 1] Scanner measurements and ATP assay results

Spheroid size and assay results from Day 2 to Day 11

Legend: HEK 293(ATP), HEK 293T(ATP), HEK 293(Area), HEK 293T(Area)

●Primary screening

The primary screening identified 39 compounds that reduced spheroid area in the 3D spheroid culture of 293T cells. Of these compounds, 4 compounds that showed selective inhibitory activity against the HEK293T cells in 3D culture were used for secondary screening.

[Figure 2] Representative images obtained on Cell³iMager for each timepoint

Scanner images of HEK293 (top row) and HEK293T (bottom row) spheroids — days 2, 4, 7, 9, 11
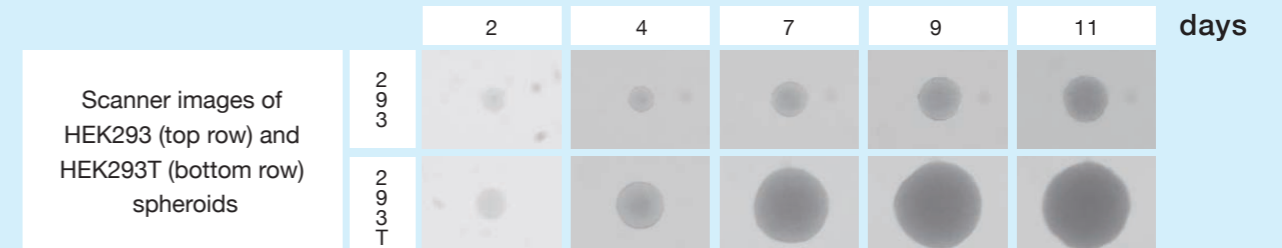
●Secondary screening

Table 1 shows results of the secondary screening. In general, the compounds that reduced spheroid area showed growth inhibition in the ATP assay. Overall, the IC50 values based on area were highly correlated with the IC50 values of the ATP assay.

[Table 1] Results of secondary screening of compounds that reduced spheroid area: IC50 (nM)

Inhibitor name	Target	3D Spheroid area		3D ATP assay	
		293	293T	293	293T
Temsirolimus	mTOR	1309	16	6667	149
PDGF receptor tyrosine kinase inhibitor IV	PDGFR	1792	57	3774	53
17–AAG	HSP90	5020	778	7299	856
Monensin	Na ionophore	847	54	440	103