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Development of novel high-throughput screening system for exploring EMT inhibitors.

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Background and Objective

Background

EMT (Epitherial-Mesenchymal transition) is a significant event in tumor metastasis and malignancy. Cancer cells are stimulated by EMT-inducible agonists from the surrounding stromal cells, and cause transformation such as weakening of cell-cell adhesion and the accompanying acceleration of cell migration and invasion. Inhibition of EMT is considered to enable controlling of malignant transformation.

Study Objective

The objective of this study was development of a high-throughput assay system of EMT inhibitor screen in 3D cell culture.

Methods

3D cell culture

We adopted 3D cell culture method on NanoCulture® Plate (SCIVAX), a scaffold type spheroid culture plate, as a culture method which can confirm spheroid morphologies.

EMT induction and inhibition

TGF-β2 and its inhibitor, SB431542, were used as an EMT inducer and as a positive control for EMT inhibitor, respectively.

Gene expression analysis

Expressions of E-cadherin, N-cadherin, vimentin, and zeb-1, represent EMT maker genes, were evaluated by qRT-PCR. TBP gene expressions was also evaluated as internal control.

Intra-spheroid hypoxia sensing

The intra-spheroid hypoxic area was visualized using Hypoxia Probe (SCIVAX).

Evaluation of the EMT status

Fluorescence intensity of the Hypoxia Probe of intra-spheroid in whole well was detected using Celigo® Imaging Cytometer (Brooks Autmation, Inc.), as an indicator for EMT induction and inhibition.

Results

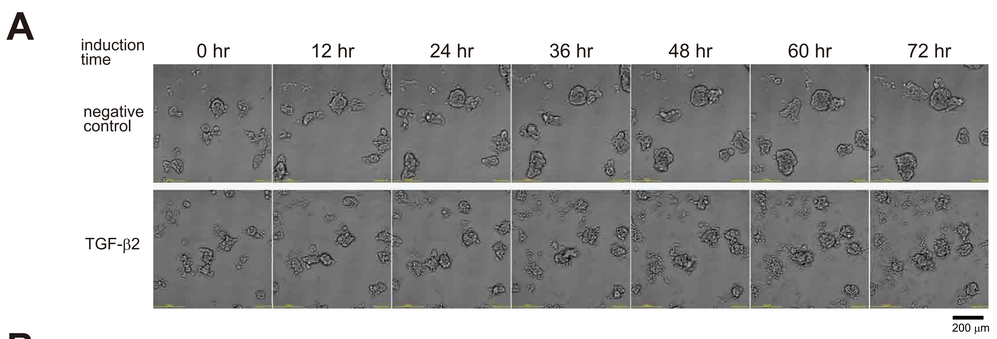


Figure 1. TGF-β2 treatment induces EMT, which is characterized by decreasing cell-cell adherence, increasing cell migration, and change of expression of EMT maker genes.
(A) A549 spheroids treated with TGF-β2 (5 ng/mL) at day 3. Time-Lapse analysis revealed migration of cells from spheroids, leads to spheroid collapse. (B) SB431542 inhibited the spheroid collapse and change of expression of EMT maker genes.

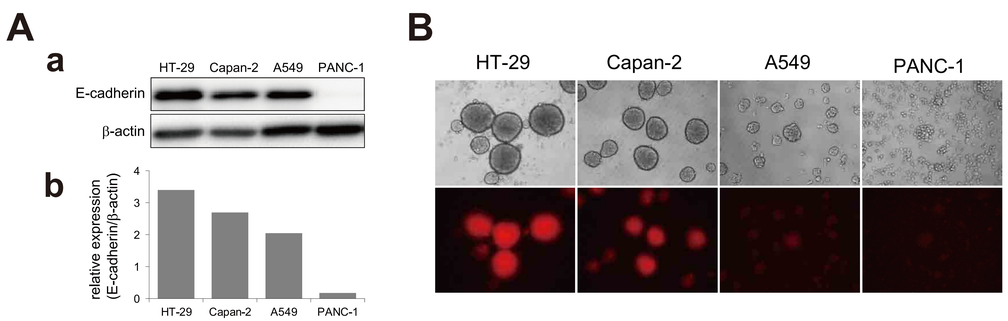
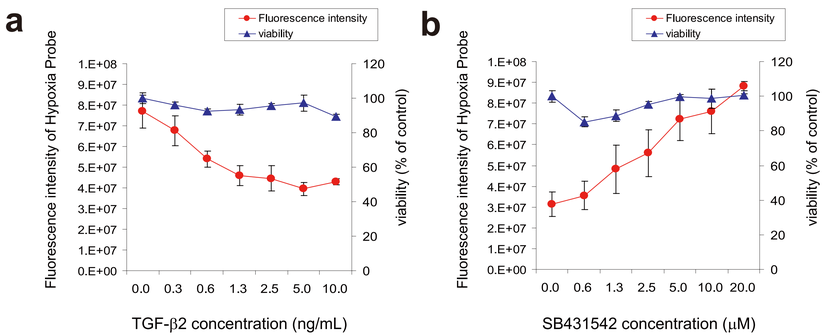


Figure 2. Fluorescence intensity of Hypoxia Probe reflects degree of cell-cell adhesion and spheroid morphology.
(A) Comparison of E-cadherin expression of epitherial cancer cell lines. (B) Cell lines form spheroids of various sizes and shapes, according to these E-cadherin expression level. Differential changes of signal intensity of Hypoxia Probe depends on the spheroid features.



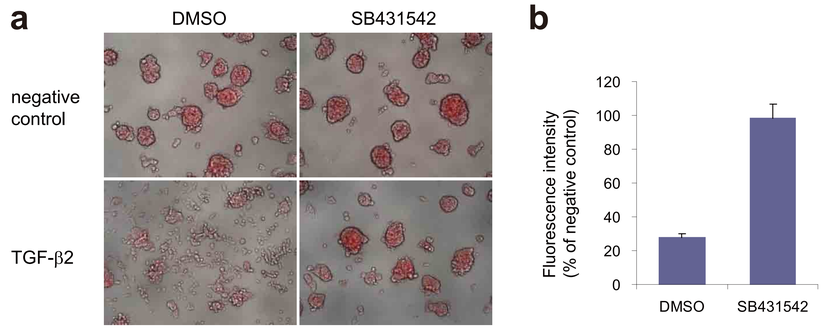


Figure 3. Degree of intra-spheroid hypoxia reflects the spheroid compactness (EMT status).
(A) Spheroids was treated with TGF-β2, or cotreated with TGF-β2 (5 ng/mL) and SB431542, for 3 days. Fluorescence intensity of Hypoxia Probe decreased according to TGF-β2 concentration, or increased according to SB431542 concentration (b). In these condition, cell viability was not affected. (B) Fluorescence intensity of Hypoxia Probe depended on the morphology of EMT-induced spheroids. The signal intensity was measured using Celigo® Imaging Cytometer.

Conclusion

- We developed unique high-throughput assay system for EMT inhibitor screen, by evaluating intra-spheroid hypoxicity as an indicator for alternation of the spheroid morphology.
- We are now running a screen for EMT inhibitor candidate compounds, by using this assay system.

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