3D culture based RNAi screening for exploring target genes of EMT inhibitor

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SCIVAX NanoCulture Plate

Abstract

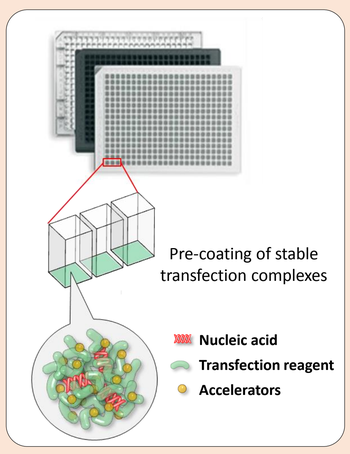
EMT (epithelial-mesenchymal transition) is characterized by the loss of epithelial characteristics and the subsequent development of a fibroblast-like phenotype. It is a significant event in tumor metastasis and malignancy. Therefore inhibition of EMT is considered to enable controlling of malignant transformation, and development of EMT inhibitors need to be addressed.

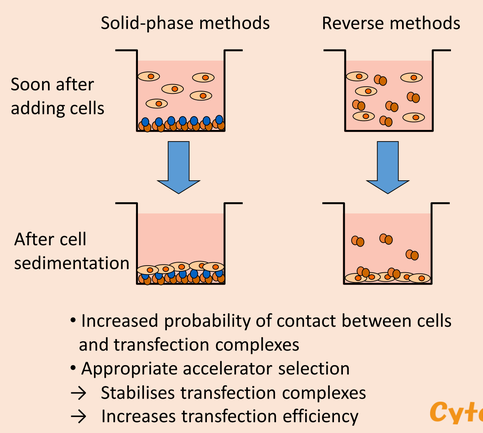
Here, we have introduced a cell based assay method for the screening of EMT inhibitor compounds by using the 3D culture plate. TGF-beta treated cells showed decreasing of cell adhesion and increasing of cell motility that lead to spheroid craps. We developed a measurement method using hypoxia probe in order to quantify more accurate data. Because degree of intra-spheroid hypoxia is correlate with spheroid cell-cell adherence. The principal of this assay is easy monitor of EMT phenomenon by using a hypoxia detecting probe reagent.

Finally we have established 3D culture based RNAi HTS technology by solid phase transfection that allows genome-wide loss-of-function screening and broadly used in the identification of genes which associated with specific EMT phenotypes. Advantage of solid phase transfection is easy to conduct the experiment, because complexes of nucleic acid and transfection reagent are pre-coated on culture surface. Another major advantage of genome-scale RNAi screening is to simultaneously interrogate thousands of genes with the ability of generating a large amount of data per experiment. Besides, we have reported several candidate target genes after a pilot screening through kinase/phosphatase siRNA library (1,280 genes).

Method: solid phase RNAi technique, and screening scheme

a





- Increased probability of contact between cells and transfection complexes
- Appropriate accelerator selection

→ Stabilises transfection complexes
→ Increases transfection efficiency

CytoPathfinder

b

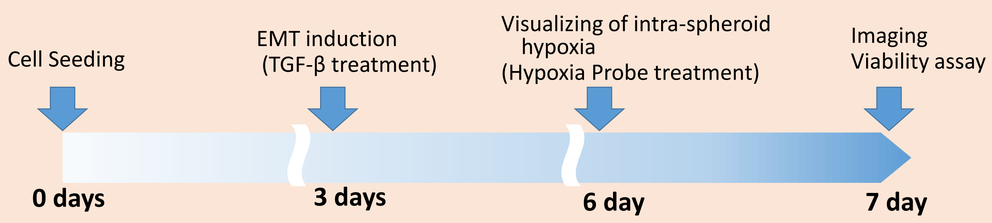


Figure 1: Cells migration and spheroid formation on NanoCulture Plate.

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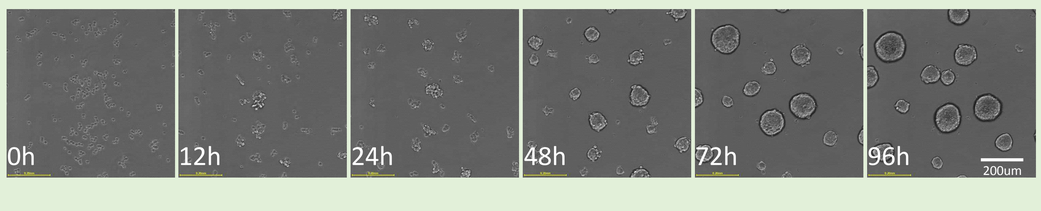

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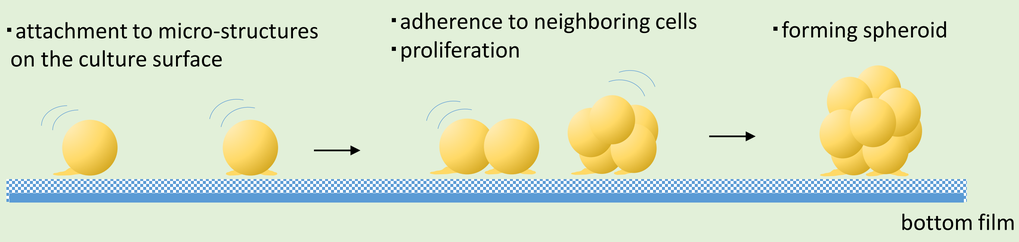
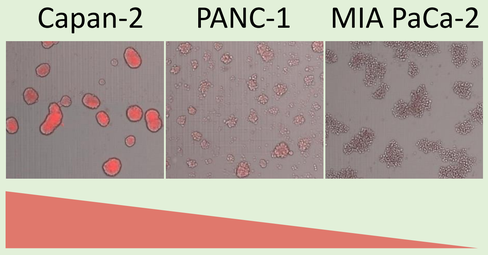


Figure 2: Degree of hypoxia inside the spheroid reflects spheroid features, i.e. size and E-cadherin expression.

c



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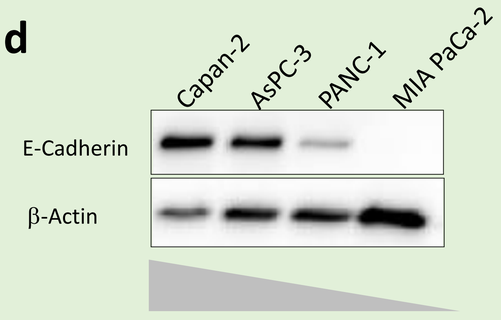

Figure 3: *In vitro* EMT model and signal of Hypoxia Probe as a surrogate marker of EMT condition

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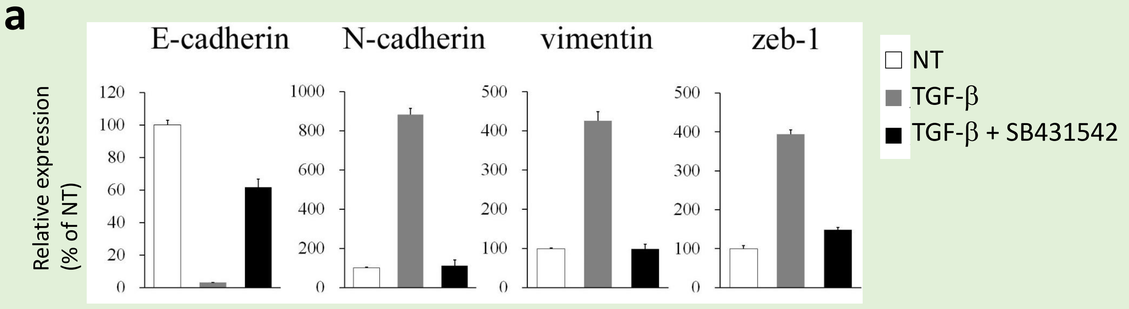

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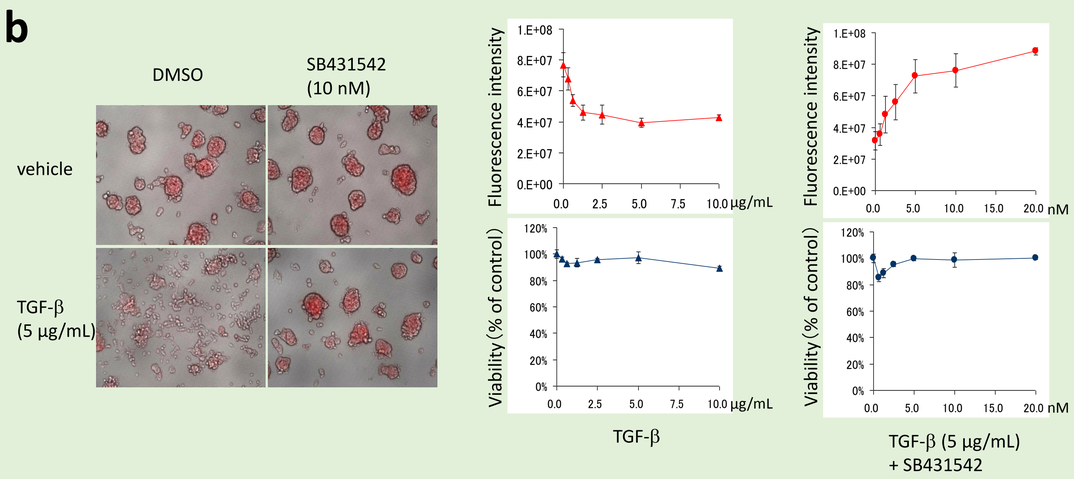

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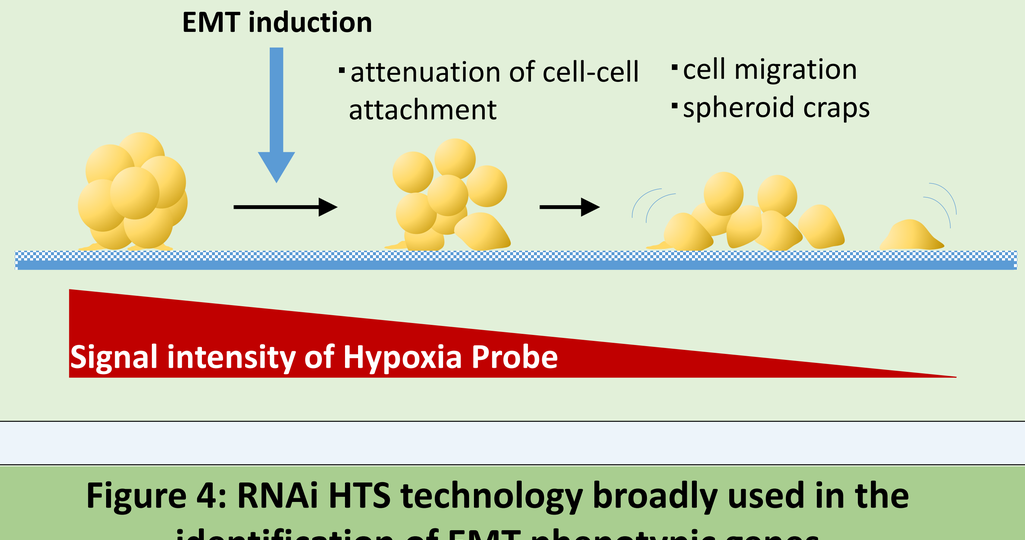

Figure 4: RNAi HTS technology broadly used in the identification of EMT phenotypic genes

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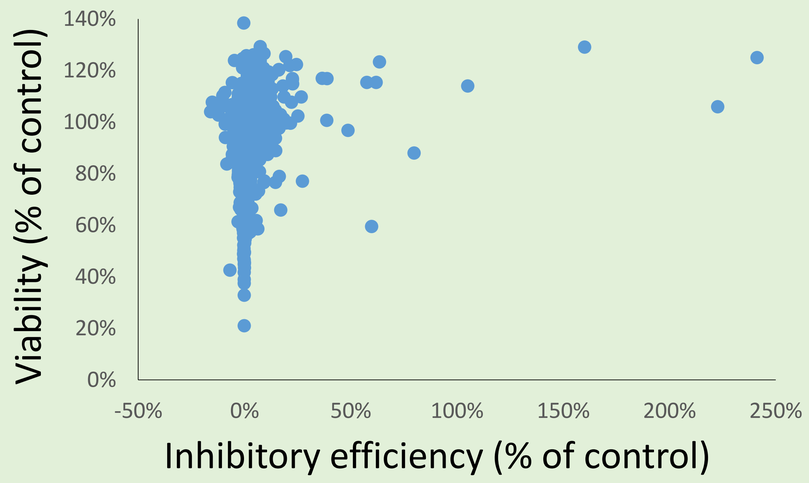

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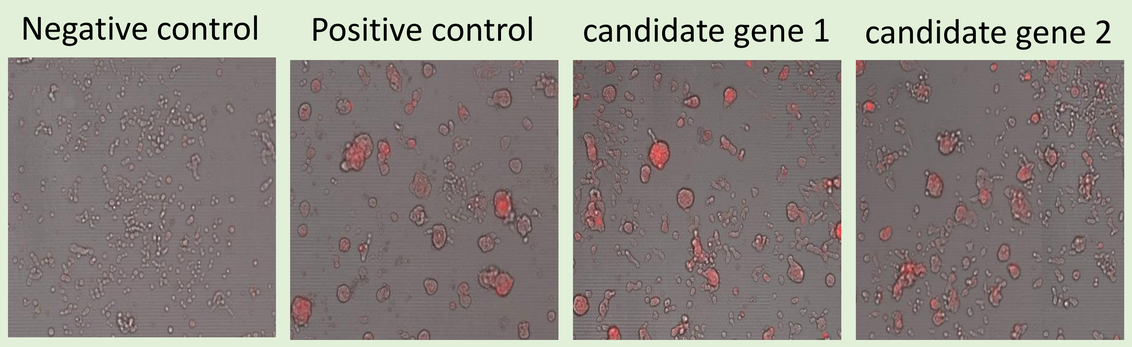

Conclusion

- We established *in vitro* EMT model by using 3D culture plate, NanoCulture Plate.
- In this model, Hypoxia Probe might be a good surrogate marker of EMT state.
- We developed novel screening system by coupling EMT model with solid phase RNAi technique.
- Several candidates of target gene for EMT inhibitor were discovered by a pilot screening.