

## ASCB

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### **A new approach for drug discovery and differentiation study using cutting-edge 3D cell culture system**

<sup>1</sup>Ana Lazic, <sup>2</sup>Fujiko Ozawa, <sup>3</sup>Manami Shimomura, <sup>4</sup>Yoshio Miyagawa, <sup>2,5</sup>Masami Hiroyama, <sup>4</sup>Nobutaka Kiyokawa, <sup>6</sup>Akihiro Umezawa, <sup>5</sup>Akito Tanoue, <sup>3</sup>Tetsuya Nakatsura, <sup>2</sup>Satoru Tanaka

<sup>1</sup> InfiniteBio <sup>2</sup> SCIVAX Corp. <sup>3</sup> Cancer Immunotherapy Proj., Invest. Treat. Div., Natl. Cancer Ctr. Hosp. East <sup>4</sup> Department of Development Biology, National Research Institute for Child Health and Development <sup>5</sup> Department of Pharmacology, National Research Institute for Child Health and Development <sup>6</sup> Department of Reproductive Biology, National Research Institute for Child Health and Development

Conventional cell-based assays commonly used in the drug discovery process are based on a monolayer (2-dimensional) cell culture system. The obtained drug efficacy data often have a poor correlation with the *in vivo* data, suggesting a need for a better *in vitro* model to mimic an *in vivo* environment. NanoCulture Plate (NCP) is a novel experimental system developed for the three-dimensional cell culture. The plate bottom has a special film having a nano-structure pattern which enables spheroid formation of various cell types.

At the molecular level, the expression levels of proteins that characterize the *in vivo* environment were altered in the spheroids compared to monolayer culture. The protein expression of HIF-1, VEGF and AKT was significantly increased in cancer cell spheroids. In addition, 3-D spheroids showed different drug susceptibility depending on the combination of specific drug and cell types compared to monolayer cells.

Furthermore, we established a method for 3D primary culture of pancreatic cancer and hepatocellular carcinoma (HCC) from clinical samples obtained from patients at National Cancer Center Hospital East, Japan. The formation of spheroid on the NCP was confirmed in approximately 90% of the samples and the excessive growth of fibroblasts was not observed. In addition, expression of HCC marker Glypican-3 (GPC3) was observed in spherical structure of the cancer cells.

NCP is also useful for spheroid formation of normal cells such as hepatocytes, adipocytes, and mesenchymal stem cells. On NCP, the characteristics of cellular differentiation in spheroid were maintained and emphasized compared to monolayer cultured cells. Hepatocytes secreted higher levels of albumin, a differentiation marker, and showed upregulation of the cytochrome P450 gene expression. Stem cells form 3D spheroids on the NCP, differentiate to adipocytes, after the induction, with a higher content of triglyceride, and highly express differentiation marker-genes.

These results suggest that spheroids on NCP have *in vivo* like characteristics. This *in vitro* 3D culture system offers a new approach for more relevant cell based assays in drug screening to obtain *in vivo* comparable results.

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