—Photogravure—

Immunocytochemical Analysis of a Three-dimensional Spheroidal Culture System

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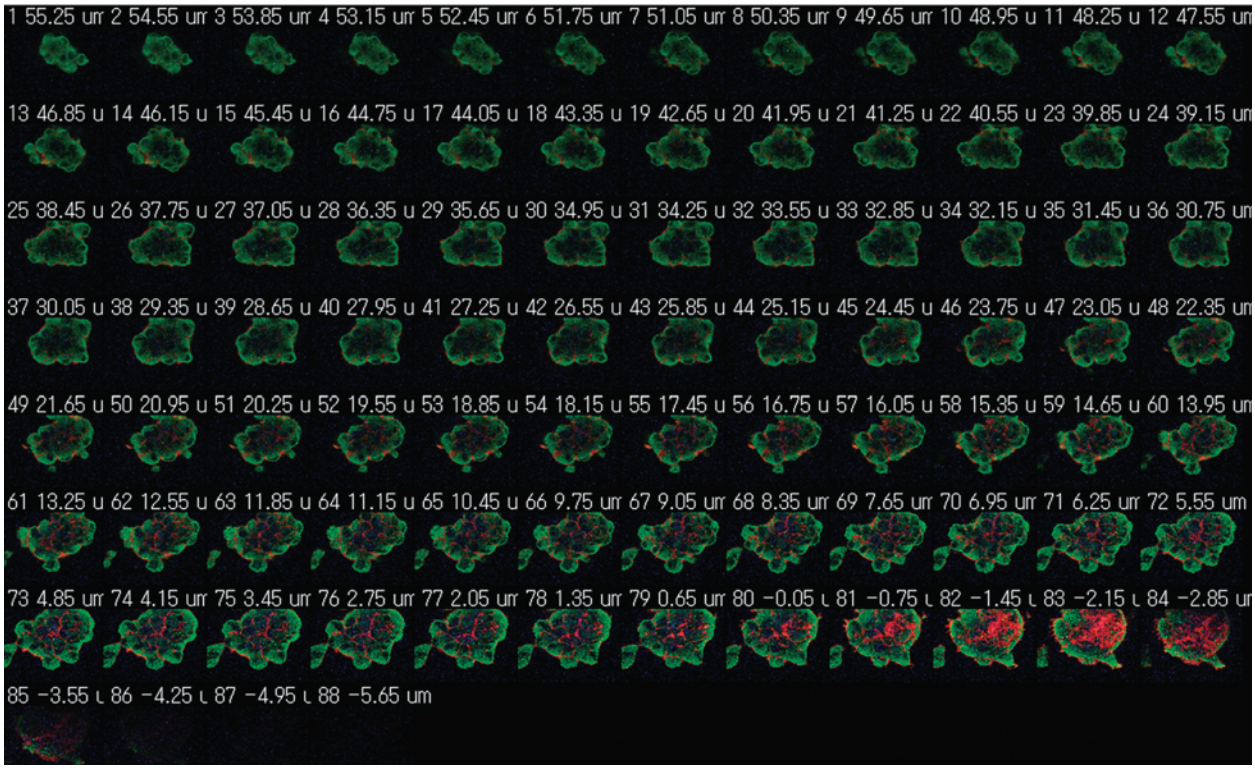

Fig. 1

Abstract

Three-dimensional (3D) cell cultures are expected to mimic in vivo environments. The NanoCulture plate (Scivax Corporation, Kawasaki) allows the formation of 3D spheroids[1,2]. The cytoskeletal proteins are considered to be important regulators of cellular morphology and structure. Among the cytoskeletal proteins, fibrillar actin (F-actin) is a main component of microfilament proteins, and α-tubulin is the major protein forming microtubules in cells. Therefore, in this study, we used NanoCulture plates to examine the expression patterns of F-actin and α-tubulin in PANC-1, a human pancreatic cancer cell line. The cells were stained with polyclonal antibodies against α-tubulin antibody and with Alexa 568-labeled phalloidin to detect F-actin. Eighty-eight horizontal images **(Fig. 1)** were obtained at 0.25-μm intervals with a confocal laser microscope (TE2000-E, Nikon Instech Co., Ltd., Tokyo). In horizontal images, the expression of F-actin was observed at the periphery of cells **(Fig. 2A–C, red)**, and strong F-actin expression was observed on the grids of the NanoCulture plate at the levels of cells nearest the plate **(Fig. 2C, arrows and inset)**. The expression of α-tubulin was observed in the cytoplasm of cells in 3D cultures **(Fig. 2A–C, green)**. Vertical images revealed that the cells piled up in 3D culture **(Fig. 2D)** and that cells formed actin-rich cell projections that attached to the surface **(Fig. 2D arrows)**. The 3D images

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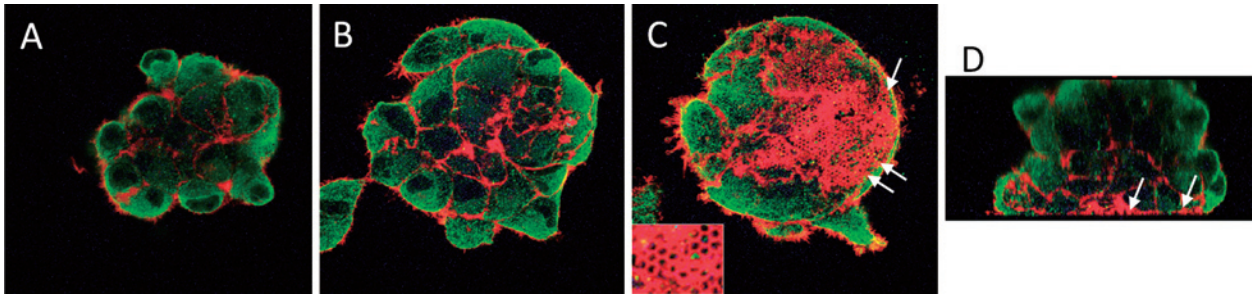

Fig. 2

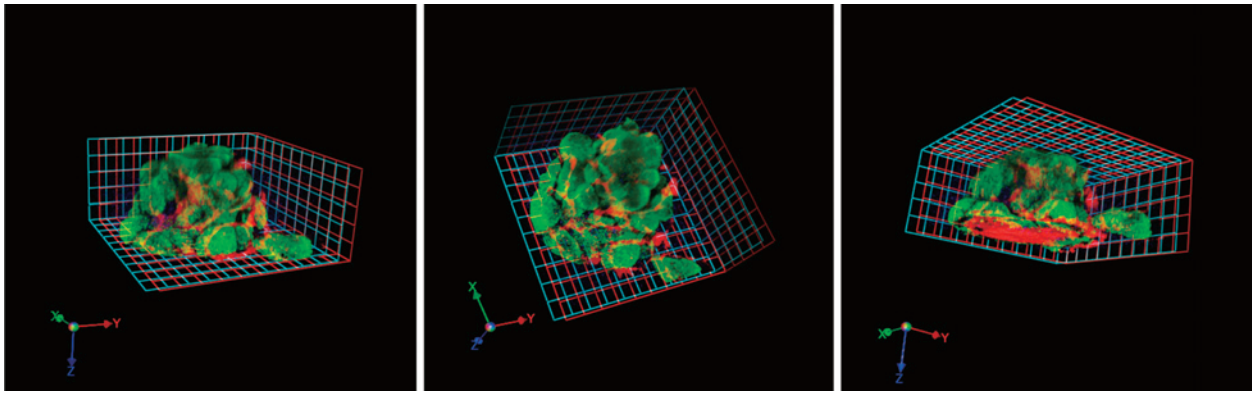

Fig. 3

(Fig. 3) revealed the different expression patterns of cytoskeletal proteins in the spheroids as well as the relationship between cell-to-cell interactions in these cytoskeletal proteins. The 3D culture enabled analysis of the morphological changes and the cell-to-cell interactions in spheroids, which cannot be observed with 2D cultures. The 3D spheroidal culture system is a useful method for cell imaging and can mimic in vivo environments.

Fig. 1 Serial images of a spheroid. Green: α-tubulin; red: F-actin; blue:4′,6-diamidino-2-phenylindole; original magnification: ×1,000.

Fig. 2 **A**, **B**, and **C**: horizontal images of a spheroid; **D**: a vertical image of a spheroid.

Fig. 3 3D images of a spheroid.

References

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2. Matsuda Y, Kawamoto Y, Teduka K, et al.: Morphological and cytoskeletal alterations of nervous system tumor cells with different culturing methods. International Journal of Oncology (in press).

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