APPLICATION NOTE

Confocal Laser Microscope A1R Si

Visualizing the dynamics of pancreatic cancer cells self-organized on the micro/nanoplate

Pancreatic cancer is known to be extremely malignant and progresses very rapidly. Yet, there are still many uncertainties about its therapeutic resistance. The research group of Yukiko Miyatake, Assistant Professor of the Graduate School of Medicine, Hokkaido University, and Kaori Kuribayashi-Shigetomi, Specially Appointed Associate Professor of the Institute for the Advancement of Higher Education, Hokkaido University, et al. developed the micro/nanoplate, a 3D cell culture device. Using cancer cells, the micro/nanoplate easily recreates microtumor cells with a structure similar to patient *in vivo* cancer cells. This application note presents 3D imaging examples of the detailed 3D structure of pancreatic cancer microtumor tissues self-organized on the micro/nanoplate. Using a Confocal Laser Microscope A1R Si, Dr. Miyatake et al. performed the study described on this application note.

The micro/nanoplate allows *in vitro* recreation of tumor tissue dynamics

Previous studies on the development of anti-cancer drugs mostly used monolayer-cultured cells and many of the insights obtained from these studies were not often consistent with the results of *in vivo* tests using animal experiments and clinical specimens. One reason for this discrepancy is believed to be that tumor cells cultured in petri dishes are completely different in form and properties from *in vivo* tumor tissues.

The micro/nanoplate developed by Dr. Miyatake, et al. allowed them to culture pancreatic cancer cells that recreated pancreatic cancer tissues similar to *in vivo* tumor tissues (see the video in Figure 1).

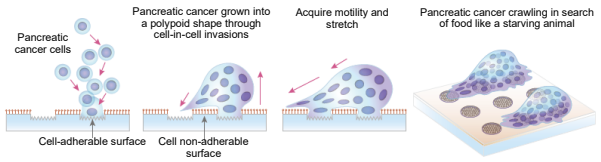

Figure 1. The figure outlines pancreatic cancer cells cultured using the micro/nanoplate

The pancreatic cancer cells cultured on the micro/nanoplate self-organize, starting with invading into neighboring cells. For the first time in history, Dr. Miyatake et al. succeeded in capturing live images of self-organized *in vitro* pancreatic cancer aggressively crawling like a starving animal.

Sample video

3D-reconstructed fluorescence images of self-organized pancreatic cancer tissues

When pancreatic tumor cells were cultured on the micro/nanoplate overnight, microtumors adhered to the micro-patterned plate and self-organized. The microtumor samples were anchored to the plate and stained for immunofluorescence microscopy. Using a spectral imaging confocal laser microscope (A1R Si), Dr. Miyatake et al. analyzed the 3D imaging data (Figures 2, 3a, and 3b).

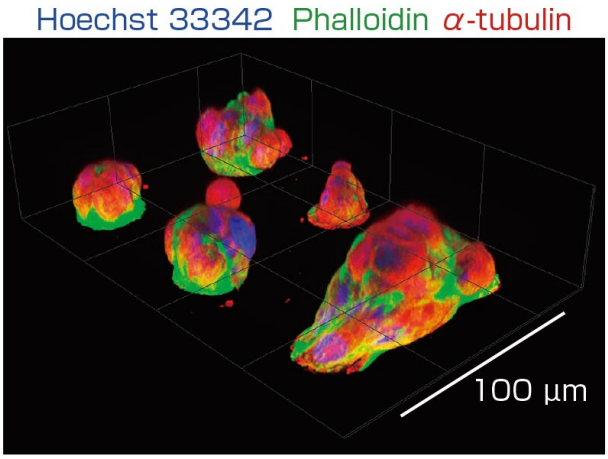

Figure 2. Pancreatic cancer microtumors self-organized on the micro/nanoplate

Label: Actin: Alexa Fluor® 488 Phalloidin, Cell nucleus: Hoechst 33342, Microtubule: (Primary Ab) Mouse anti-human α-tubulin, (Secondary Ab) Alexa Flour® 488/594 conjugated goat polyclonal antibody
Objective: CFI Plan Apochromat VC 60XC WI (oil)
Measurement condition: Microtumors formed on the microfabricated plate are anchored and immunostained followed by confocal observation.
Image: Dr. Yukiko Miyatake, Department of Pathology, Graduate School of Medicine, Hokkaido University

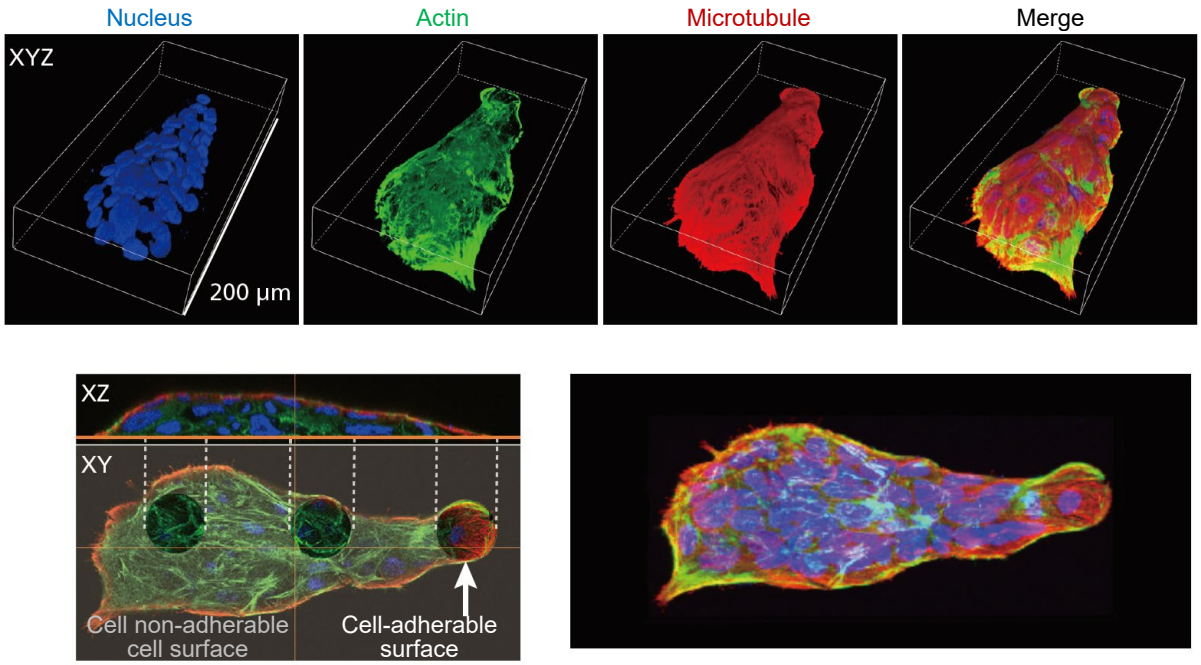

Figure 3a. Pancreatic cancer microtumor consisting of approximately 80 pancreatic cancer cells

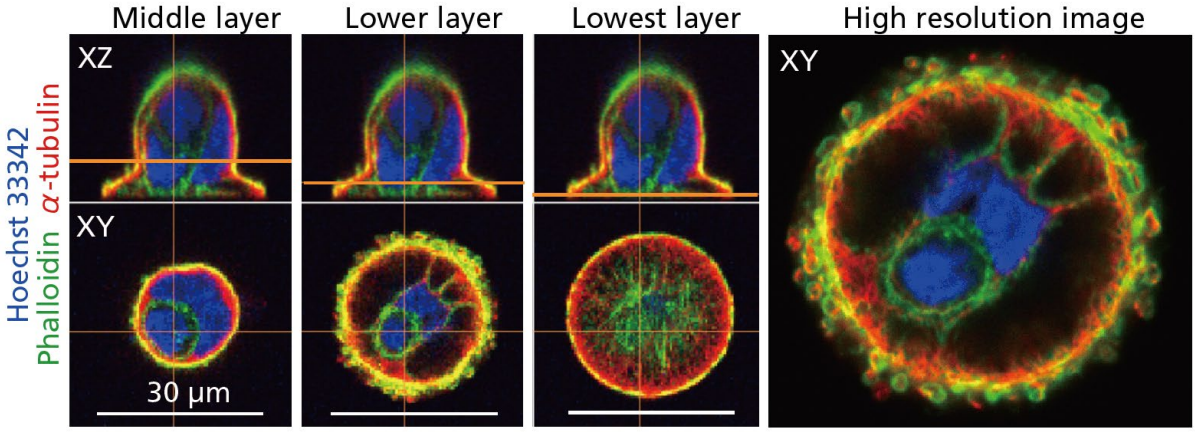

Figure 3b. Pancreatic cancer microtumor tissue with a tubular structure

Result

Observations with the Nikon Confocal Laser Microscope A1R Si allows improved molecular and physiological analysis at the micro-tissue level. The 3D analysis revealed that polypoid pancreatic cancer microtumors that self-organized and stretched in the Z-axis direction formed cell-in-cell structures and tubular structures. Furthermore, some of the mature pancreatic cancer microtumors exhibited tissue motion polarity, indicating they had acquired multicellular 3D collective cell motility.

Summary

The micro/nanoplate allows *in vitro* dynamics analysis of pancreatic cancer at the tumor tissue level. This development is expected to help understand new microtissue-level mechanisms, including the formation and invasion of pancreatic cancer, and contribute to the development of new anti-cancer drugs without animal experiments.

References

Miyatake Y, Kuribayashi-Shigetomi K et al.
Visualising the dynamics of live pancreatic microtumours self-organised through cell-in-cell invasion
Scientific Reports, 14054, 2018

Product data

Confocal Laser Microscope A1R HD25

Advanced high-speed, high-resolution resonant scanner that achieves a large field of view and reduced photobleaching and phototoxicity to live cells

- High speed: up to 720 FPS
- High resolution: up to 1K (1024 x 1024 pixels)
- High throughput: Super large 25 mm field of view