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Morphological and cytoskeletal changes of pancreatic cancer cells in three-dimensional spheroidal culture

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Abstract Three-dimensional (3D) cell cultures are expected to mimic in vivo environments. We used a NanoCulture plate to determine the spheroid-forming ability of pancreatic ductal adenocarcinoma (PDAC) cell lines and compared the morphology and expression of cytoskeletal proteins of PDAC cells to those in two-dimensional (2D) cultures. All examined PDAC cells grew as monolayers in 2D culture. PANC-1 and KLM-1 formed spheroids in 3D culture, but PK-45H and MIAPaCa-2 did not. Strong expression of F-actin was observed in the cells attached to the surface of the plate, which formed cell projections in 3D culture. F-actin was detected on the grids of the NanoCulture plate in PANC-1 cells but not in PK-45H. The levels of tubulin expression in cells were higher in 3D culture than in 2D culture. The expression level of E-cadherin mRNA in PANC-1 and KLM-1 was higher than that in PK-45H and MIAPaCa-2. In conclusion, PDAC cells showed morphological changes, spheroid formation, and alterations of cytoskeletal proteins in 3D culture. E-cadherin might be one of the key molecules involved in spheroid formation of PDAC cells. The 3D spheroidal culture system was a useful method for cell imaging with contrast-phase microscopy and confocal microscopy.

Key words Pancreatic cancer · Three-dimensional spheroidal culture · Actin · Tubulin

Introduction

Pancreatic ductal adenocarcinoma (PDAC) causes high mortality as a result of rapid progression and a high incidence of metastases and peritoneal dissemination.[1,2] In spite of recent medical progress, there are few or no effective therapies for advanced PDAC. Conventional and effective experimental methods are needed to examine the biological behavior of PDAC and develop diagnostic and therapeutic strategies for PDAC.

Traditional cancer cell culture studies using two-dimensional (2D) conditions have many advantages. Nevertheless, cancer cells grown on flat 2D culture systems might differ in their morphology, cell-to-cell and cell-to-matrix adhesions, and cellular differentiation from those growing in vivo.[3] On the other hand, animal studies are known to be definitive methods to clarify the effects of specific molecules or chemicals on cancer cells. However, it is difficult to monitor morphological changes of cancer cells and the alteration of expression or activation levels of cell signaling pathways of the cells in animal models.

Three-dimensional (3D) cell culture systems have been expected to mimic in vivo environments.[4–6] Many kinds of 3D culture systems including spheroid culture, ex vivo culture,[7] multilayered postconfluent cell culture,[8] the growing of cells in extracellular matrix gel,[9] and co-culture with fibroblasts or vascular endothelium[10] have been reported. Spheroid cultures are only used for cell lines that agglutinate and make colonies without attachment to the culture plates. Ex vivo culture makes use of tissues harvested in vivo, and then the tissues are cultured in vitro. This approach is particularly effective for short-time analysis. In multilayered postconfluent cell cultures, the cells are grown in V-bottomed microtiter plates to form multilayers. Growing cells in extracellular matrix gel allows cell differentiation and is effective in analyses of cell-to-matrix interactions. Co-culturing the cells with fibroblasts or vascular endothelium provides us basic information about cell-to-cell interactions. Numerous genes and proteins were found to be expressed differently in melanoma and hepatoma cell lines between 2D and 3D cell culture models.[11,12] The cell morphology and activated patterns of cell signaling pathways of prostatic cancer cells in 3D culture are often similar to those of the cells in vivo.[13] Several metabolic enzymes such as cytochrome P450 are activated in 3D culture. Therefore, 3D culture has been used in new drug research and development.[8,14] Furthermore, 3D models permit more

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convenient and rapid experiments than animal models. In vitro 3D models are expected to be useful tools for the field of molecular biology and provide a third approach to explain the mechanisms of disease or aid in drug development, in addition to traditional 2D cell culture and animal models.[15]

In spheroid cultures, several ways of making spheroids in vitro have been developed.[4,16] In traditional methods, plastic culture plates coated with poly-(2-hydroxyethyl methacrylate) solution to prevent cell attachment can enable spheroid formation.[11] 3D matrix scaffolds, which have been generated from purified molecules such as collagen I and synthetic biomaterials, have also been used to form cell spheroids.[17] The recently developed NanoCulture plate, which has a specific microsquare pattern on the bottom of the plate, allows the formation of spheroids without the need for any gel, matrix, or scaffold. On the finely structured plate, which provides a foothold for the cells, 3D spheroids can form. Furthermore, NanoCulture plates permit the staining and observation of cells without removing them from the plate. In the present study, we used the NanoCulture plate to determine the spheroid-forming ability of PDAC cell lines. Morphological changes and different expression patterns of cytoskeletal proteins of PDAC cells in 2D and 3D cell cultures were analyzed with phase-contrast microscopy and confocal laser scanning microscopy.

Materials and methods

Materials

The following materials were purchased: NanoCulture 96-well plate (NCP-L-MS) and NanoCulture medium (NCM-M) from Scivax Corporation (Kanagawa, Japan); 35-mm glass-bottom dish from Matsunami Glass (Osaka, Japan); Alexa 568-labeled phalloidin and Tubulin Tracker Green from Invitrogen Corporation (Carlsbad, CA, USA), and Vectashield H-1200 containing 4′,6-diamidino-2-phenylindole-2HCl (DAPI) from Vector Laboratories (Burlingame, CA, USA); FastPure RNA Kit (Takara Bio, Japan); High Capacity cDNA Reverse Transcription Kit, TaqMan Gene Expression Master Mix, and TaqMan Gene Expression Assays for E-cadherin and 18 S rRNA from Applied Biosystems (Carlsbad, CA, USA). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Pancreatic cancer cell lines

PDAC cell lines containing PANC-1, MIA PaCa-2, KLM-1, and PK-45H were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The cells were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) at 37°C under a humidified 5% CO_2 atmosphere.

Cell treatments

For 2D cell culture, the PDAC cells were suspended in RPMI 1640 medium containing 10% FBS and plated in 35-mm glass-bottom dishes at a density of 1×10^5 cells/2 ml. The cells were also suspended in 0.1 ml NanoCulture medium containing 10% FBS and plated in the NanoCulture plate (1×10^4 cells/0.1 ml) for 3D cell culture. The dishes and plates were incubated for 72 h at 37°C in a humidified 5% CO_2 atmosphere.

Labeling for tubulin and filamentous (F)-actin

Tubulin Tracker Green was used following the manufacturer's instructions. Briefly, 250 nM staining solution was added to the medium, which was then incubated for 30 min at 37°C in a humidified 5% CO_2 atmosphere. The staining solution was removed, and the cells were rinsed three times with phosphate-buffered saline. Then, the cells were fixed in 4% paraformaldehyde solution for 15 min at room temperature. The fixed cells were treated with Alexa 568-labeled phalloidin (1:50 dilution) for 30 min and then mounted with Vectashield H-1200 containing DAPI. The tubulin and F-actin expression levels of PDAC cells were observed using a Digital Eclipse C1 TE2000-E confocal microscope with a blue diode (excitation, 405 nm; emission, 450/35 nm), an argon laser (excitation, 488 nm; emission, 515/30 nm), and helium-neon lasers (excitation, 543 nm; emission, 605/75 nm). Fluorescence images were acquired by a Digital Eclipse C1 TE2000-E confocal microscope and analyzed using the control software EZ-C1 (Nikon Insteck, Tokyo, Japan). The confocal settings including the laser power and detector sensitivity were unchanged during the acquisition of all images. Images were collected at 0.5-μm intervals with a laser to create a stack in the Z-axis, and were thus used to build up a three-dimensional image using Volocity (Improvision, Coventry, England). A phase-contrast image of the cells was observed using a Nikon Eclipse TE2000-U microscope.

Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

To obtain RNA samples, the PDAC cells were suspended in RPMI 1640 medium containing 10% FBS and plated in 35-mm dishes at a density of 2.5×10^5 cells/2 ml for 48 h. Total RNA extraction from cells was purified with FastPure RNA Kit. One microgram of total RNA sample was used for reverse transcription (RT) with a High Capacity cDNA Reverse Transcription kit, following the manufacturer's protocol. Quantitative RT-PCR (qRT-PCR) for E-cadherin and 18 S rRNA were performed with the StepOne real-time PCR system using specific primers and a TaqMan probe for E-cadherin (Hs01013953_m1) and 18 S rRNA (Hs99999901_s1). PCR was carried out in 20-μl reaction mixtures containing 10 μl 2× TaqMan Gene Expression Master Mix, 2 μl template cDNA, and 1 μl TaqMan Gene Expression Assays. Cycling conditions were as follows: 20 s at 95°C, and then 40 cycles of 1 s at 95°C followed by 20 s at 60°C.

qRT-PCR results were expressed as E-cadherin/18 S rRNA, as an internal standard concentration ratio. Gene expression levels were measured in triplicate.

Results

Phase-contrast images

PDAC cells including PANC-1, KLM-1, PK-45H, and MIAPaCa-2 grew as monolayers and exhibited a sheet-like appearance, with spindle to polygonal cell shapes, in 2D glass-bottom dishes (Fig. 1A,C,E,G, respectively). KLM-1 cells showed tighter cell-to-cell adhesion than other cell lines (Fig. 1C). On the other hand, PANC-1 and KLM-1 cells formed spheroids in NanoCulture plates (Fig. 1B,D, respectively). Most PK-45H and MIAPaCa-2 cells showed round shapes and did not form spheroids in NanoCulture plates (Fig. 1F,H, respectively).

Expression patterns of F-actin and tubulin

The cytoskeletal proteins are considered to be important regulators of cellular morphology and structure.[18,19] PANC-1 cells formed spheroids, but PK-45H cells did not form them in NanoCulture plates. Therefore, we compared the expression patterns of cytoskeletal proteins in PANC-1 cells and PK-45H cells under 2D and 3D culture conditions. F-actin, which forms microfilaments, was strongly expressed at the periphery and cell membranes of the cells on horizontal plane at an intermediate level on the Z-axis in 2D culture (Figs. 2A and 3A, red color). Actin stress fibers in the cytoplasm were detected at significant levels at the lower levels of the Z-axis for PANC-1 and PK-45H cells in 2D culture (Figs. 2B and 3B, arrowheads). In 3D culture, the expression of F-actin was observed at peripheral regions and cell membranes of the cells on a horizontal plane at an intermediate level on the Z-axis (Figs. 2D and 3D, red color). Strong F-actin expression in PANC-1 cells was observed on the grids of the NanoCulture plate, and the positive staining of F-actin appeared as meshes at the level of cells nearest the plate (Fig. 2E, arrowhead and inset). In PK-45H cells, F-actin was prominently localized at the level nearest the plate, but F-actin was not present on the grids (Fig. 3E, arrowhead and inset). In 2D and 3D cultures, the expression pattern of tubulin, which forms microtubules, was observed in the perinuclear cytoplasm of PANC-1 and PK-45H cells as filamentous or net-like structures, but not in the periphery of the cells (Figs. 2 and 3, green color). The expression levels of tubulin in PANC-1 and PK-45H cells were higher in 3D culture than in 2D culture, but the expression patterns in the cells were not altered.

Images were collected at 0.5-μm intervals with a laser to create a stack in the Z-axis, and then they were used to build up a vertical image using the Volocity software for image analysis. Vertical images revealed that PANC-1 and PK-45H cells formed a monolayer in 2D culture (Figs. 2C, 3C). On the other hand, PANC-1 cells were piled up and formed spheroids in 3D culture (Fig. 2F). PK-45H cells were not piled up in 3D culture, but the cells formed a thicker layer than those in 2D culture (Fig. 3C,F). In 3D culture, PANC-1 and PK-45H cells formed actin-rich cell projections that attached to the surface (Figs. 2F and 3F, arrows), but cell projections were not clearly detected in 2D culture (Figs. 2C, 3C). We also performed immunofluorescent analyses using KLM-1 and MIAPaCa-2, which showed morphological changes similar to PANC-1 and PK-45H cells, respectively (data not shown).

Expression level of E-cadherin mRNA in PDAC cells

To clarify the underlying mechanisms between spheroid-forming ability and cell-to-cell attachment, we examined the expression level of E-cadherin in PDAC cells by qRT-PCR. The expression level of E-cadherin mRNA in KLM-1 cells was the highest, and PANC-1 cells showed the second-highest level of E-cadherin mRNA (Fig. 4). PK-45H and MIAPaCa-2 cells showed lower expression levels of E-cadherin mRNA than those of KLM-1 and PANC-1 cells.

Discussion

In the present study, PANC-1 and KLM-1 cells formed spheroids in 3D culture, but PK-45H and MIAPaCa-2 cells did not. To clarify the changes in cell morphology, we analyzed the expression of cytoskeletal proteins, including F-actin and tubulin. Actin stress fibers were clearly observed at the level of the cells nearest the plate in 2D culture, whereas the level of stress fiber formation was lower in the 3D culture system. Stress fibers are anchored to the cell membrane, and the sites where this anchoring occurs are also frequently connected to structures outside the cells. Therefore, changes in cell-to-cell interaction and cell-to-extracellular matrix interaction between 2D and 3D cultures might alter the expression of actin stress fibers. Interestingly, F-actin was strongly expressed in the area adhered to the plate in 3D culture. Strong F-actin expression was observed on the grids of the NanoCulture plate in PANC-1 cells, although this was not the case in PK-45H cells on the grids. Vertical images revealed that both PDAC cells formed cell projections that attached to the surface. Actin-rich membrane projections are called filopodia, lamellipodia, or invadopodia, and these actin structures have important roles in cell migration, invasion, and attachment.[18–20] Therefore, the different patterns of F-actin expression on the grid of PANC-1 cells and PK-45H cells might be the cause of differences in spheroid formation.

The levels of tubulin expression in PDAC cells were higher in 3D culture than in 2D culture, but the expression patterns in the cells were not altered. This result agrees with the finding of a previous study that the level of expression of tubulin beta-2 chain was higher in 3D culture than in 2D culture, as determined by proteomic analysis.[21] Tubulin forms microtubules and centrosomes, and is essential for many vital cellular processes such as intracellular transport,

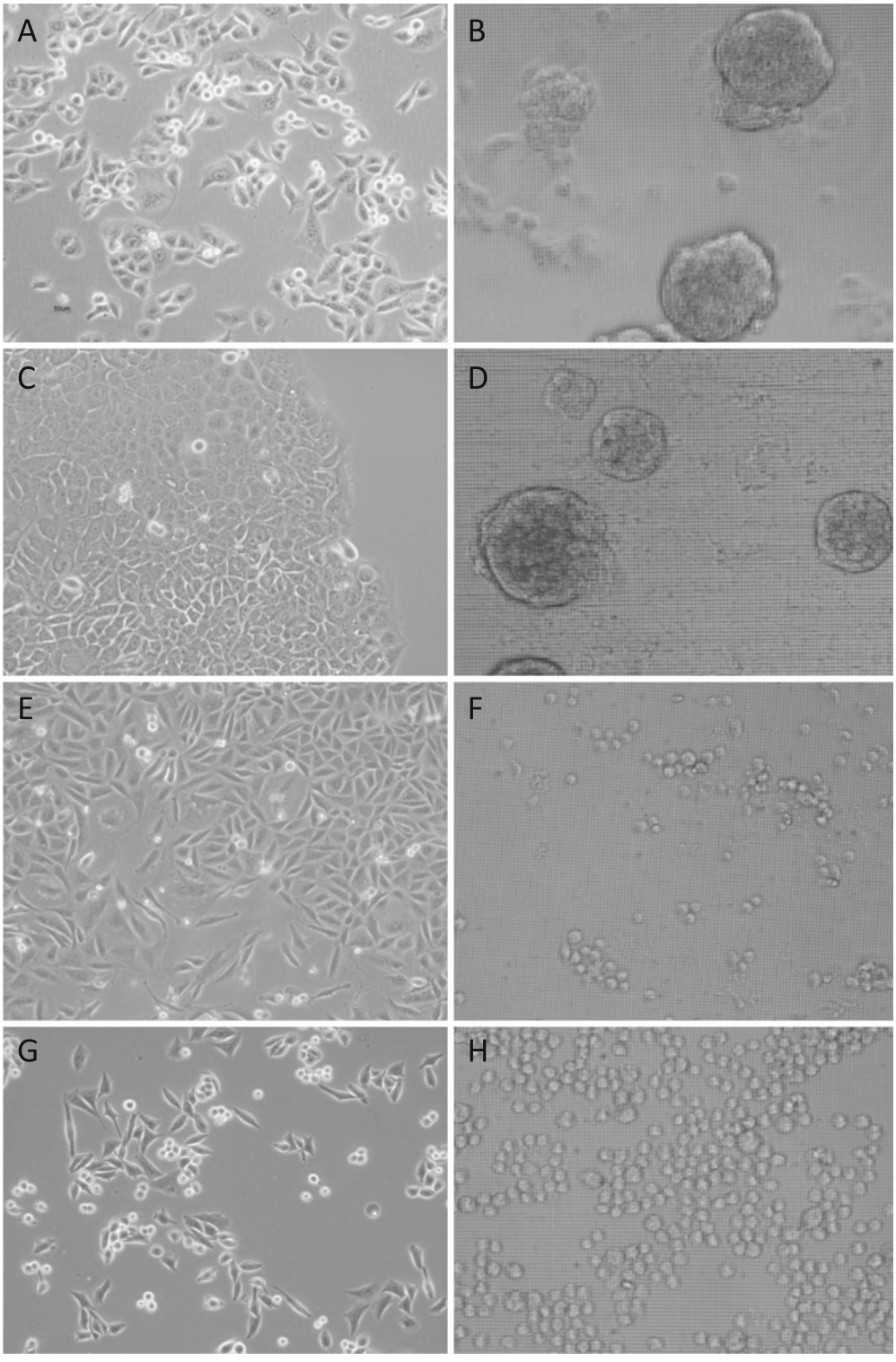

Fig. 1. Phase-contrast images of pancreatic cancer cells in two-dimensional (2D) and three-dimensional (3D) cultures. All the pancreatic ductal adenocarcinoma (PDAC) cells formed monolayers that exhibited sheet-like appearances, and the cells showed spindle-like to polygonal shapes in 2D culture (**A**, **C**, **E, G**). PANC-1 cells and KLM-1 cells formed large spheroids (**B**, **D**), but PK-45H and MIAPaCa-2 cells did not show spheroid formation in 3D culture (**F**, **H**). **A**, **B**, PANC-1; **C**, **D**, KLM-1; **E, F**, PK-45H; **G**, **H**, MIAPaCa-2. ×100

metabolism, and cell division. Therefore, the increased level of tubulin expression observed in 3D culture might be important in terms of cellular biology in vitro.

Previous studies have been showed that many genes and proteins were expressed differently in the same cell lines between 2D and 3D cell culture models,[11,12] and activated patterns of cell signaling pathways of the cells in 3D culture are considered to be similar to those of the cells in vivo.[13] The morphological changes and cell-to-cell and cell-to-extracellular matrix interactions might cause distinct expression of genes and proteins. To clarify the underlying mechanisms between cell-to-cell interactions and spheroid-forming ability, we analyzed the expression level of E-cadherin. In this study, E-cadherin expression in PDAC

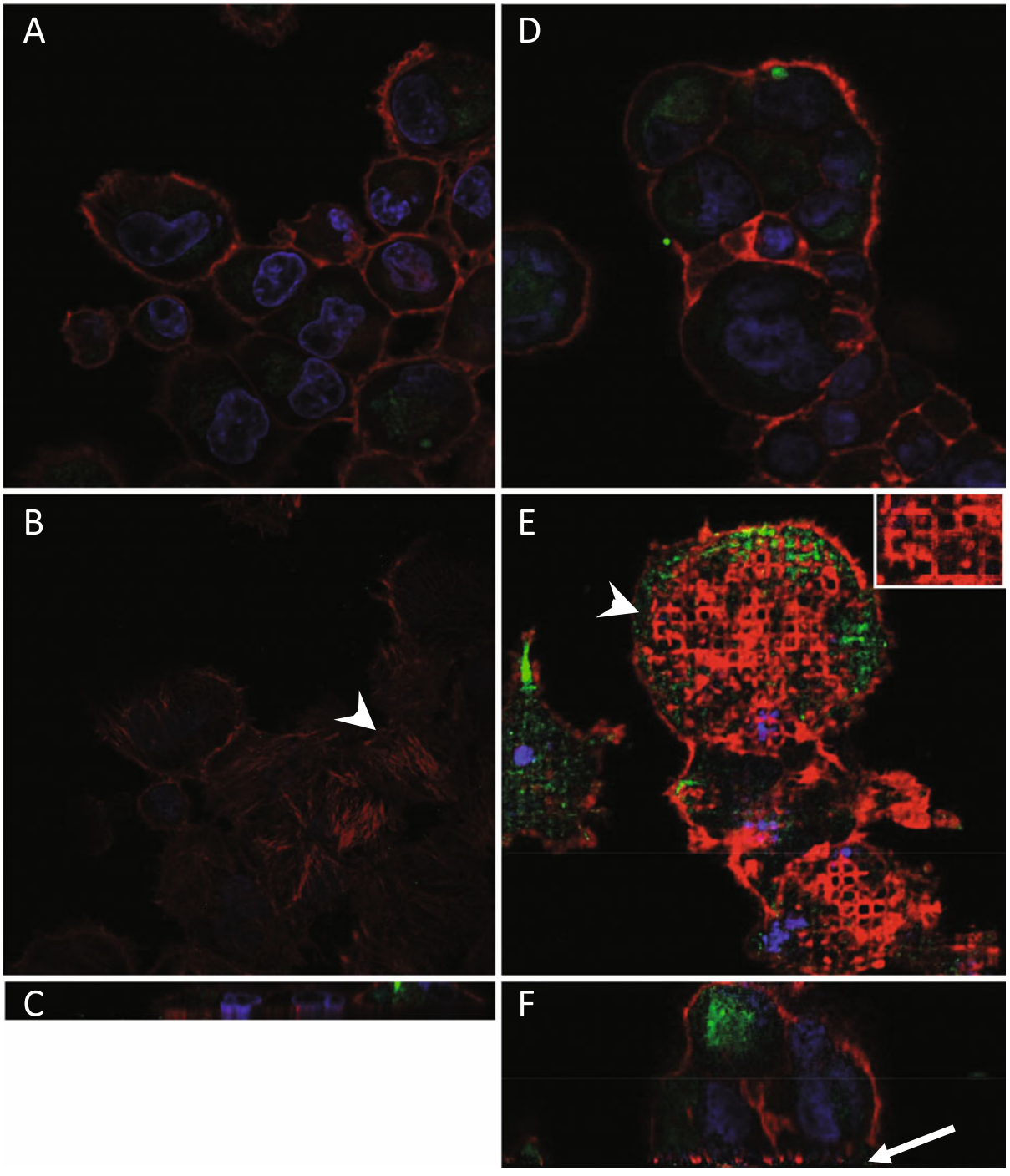

Fig. 2. Expression of F-actin and tubulin in PANC-1 cells in 2D and 3D cultures. In 2D culture, F-actin was observed in the cell membrane at the intermediate level of the Z-axis (**A**), and the stress fibers were prominently localized in the cells at the lower levels of the Z-axis (**B**, *arrowhead*). In 3D culture, the expression of F-actin was observed at peripheral regions and cell membranes of the cells at the intermediate level of the Z-axis (**D**). Strong F-actin expression in PANC-1 cells was observed on the grids of the NanoCulture plate in the cells nearest the plate (**E**, *arrowhead* and *inset*). The expression of tubulin was observed in the perinuclear cytoplasm of PANC-1 cells in 2D and 3D cultures (**A**, **D**). Vertical images revealed that cells formed monolayers in 2D culture, but piled up in 3D culture (**C** and **F**, respectively), and cells formed actin-rich cell projections that attached to the surface (**F**, *arrow*). Merging of immunofluorescence of tubulin and F-actin (tubulin, *green*; F-actin, *red*; DAPI, *blue*). Images for intermediate level of the Z-axis (**A**, **D**), images for level of the Z-axis closest to the plate (**B**, **E**), and vertical images reconstructed using computer software (**C**, **F**). ×1000

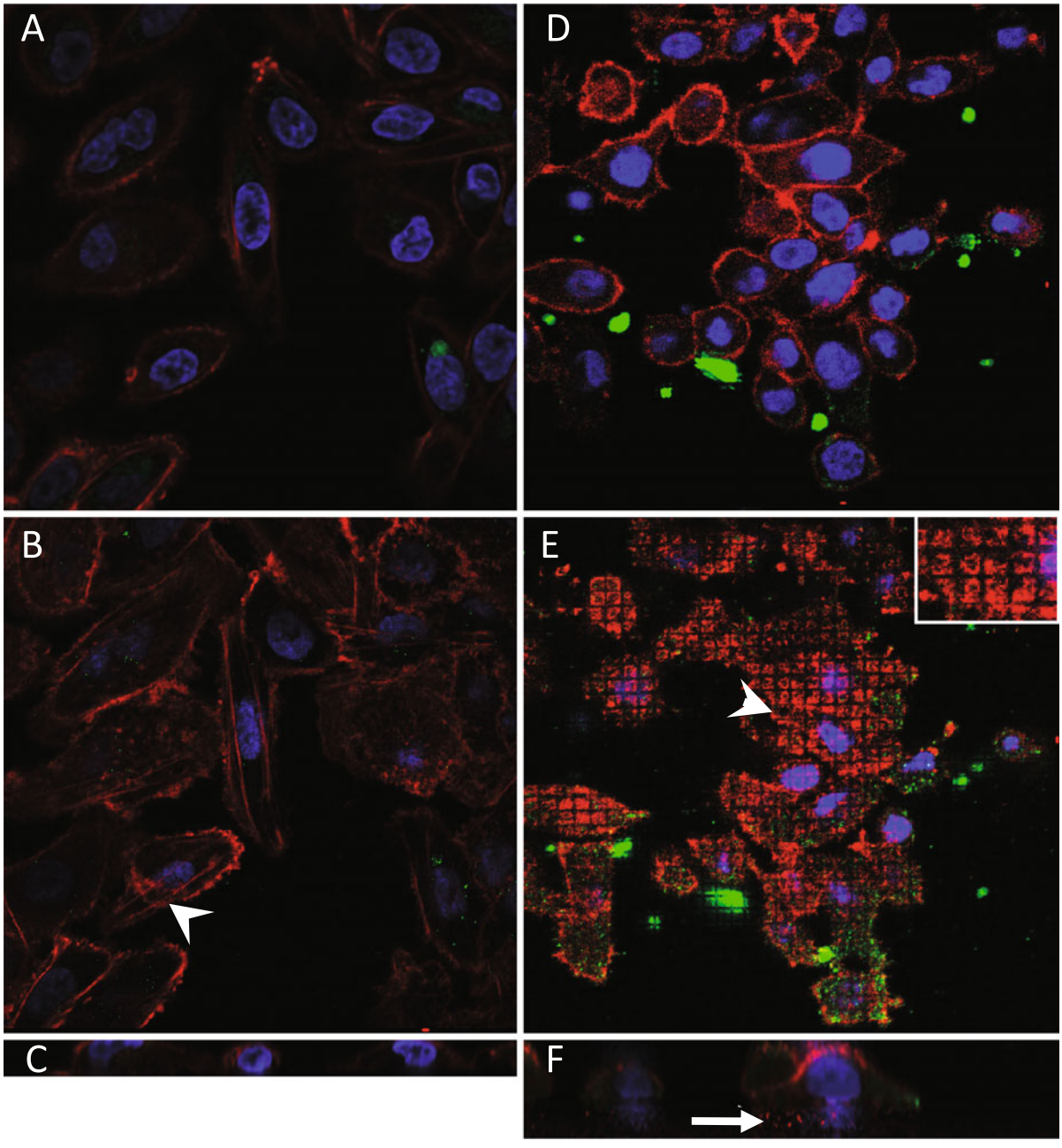

Fig. 3. Expression of F-actin and tubulin in PK-45H cells in 2D and 3D cultures. In 2D culture, F-actin was observed in the cell membrane at the intermediate level of the Z-axis (**A**), and the stress fibers were prominently localized in the cells nearest the plate (**B**, *arrowhead*). In 3D culture, the expression of F-actin was observed at peripheral regions and cell membranes of the cells at the intermediate level of the Z-axis (**D**). Strong F-actin expression in PK-45H cells was observed nearest the plate, but it was not detected on the grids of the NanoCulture plate (**E**, *arrowhead* and *inset*). The expression of tubulin was observed in the cytoplasm of PK-45H cells in 2D and 3D cultures (**A**, **D**). Vertical images revealed that the PK-45H cells formed monolayers in 2D and 3D cultures (**C**, **F**). In 3D culture, cells were piled up and formed cell projections that attached to the surface (**F**, *arrow*). Merging of immunofluorescence of tubulin and F-actin (tubulin, *green*; F-actin, *red*; DAPI, *blue*). Images for intermediate level of the Z-axis (**A**, **D**), images for level of the Z-axis closest to the plate (**B**, **E**), and vertical images reconstructed using computer software (**C**, **F**). ×1000

cells was positively correlated with spheroid-forming ability. PANC-1 and KLM-1 cells, which express higher E-cadherin, showed an epithelioid-like appearance in 2D culture and formed spheroids in 3D culture. PK-45H and MIAPaCa-2, expressing a low level of E-cadherin, showed a mesenchymal-like morphology in 2D culture and did not form spheroids in 3D culture. Each cell line has a characteristic differentiation, and it may cause the differences in spheroid-forming ability.

In the present study, a newly developed 3D cell culture system using NanoCulture plates was found to be as useful for cell imaging with contrast-phase microscopy and confocal microscopy as 2D culture. The 3D culture enabled analysis of the morphological changes of cell process formation

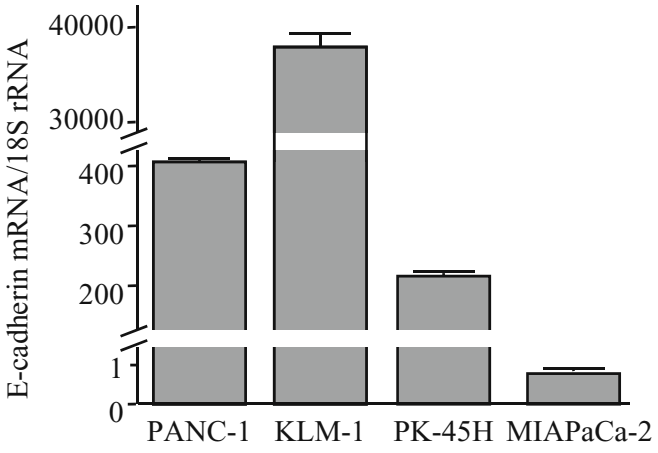

Fig. 4. Expression level of E-cadherin mRNA in PDAC cells. The expression level of E-cadherin mRNA in KLM-1 cells was the highest, and PANC-1 cells showed the second-highest level of E-cadherin mRNA. PK-45H and MIAPaCa-2 cells showed lower expression levels of E-cadherin mRNA than KLM-1 and PANC-1 cells

and cell-to-cell interaction inside spheroids, which cannot be observed with 2D culture. Traditional 3D culture systems with a low-adhesion plate require a complicated process for cell staining such as cases where the spheroids were collected and then embedded in paraffin. By contrast, the NanoCulture plate is easy to use, because we can seed, culture, and stain the cancer cells using the conventional 2D culture techniques without the need for complicated processes. Therefore, this in vitro 3D model allows easy morphological analysis in an environment similar to that in vivo.

The spheroids on the NanoCulture plate can migrate while remaining attached to the plates and viable, whereas spheroids in poorly attaching products cannot attach to plates, and the central part of the spheroid is thus nonviable.[11] It is unsurprising that there are many contradictory findings among the several 3D culture models. Further study is needed to clarify the characteristics of each 3D culture model and find the best way to reproduce the in vivo environment.

In conclusion, PANC-1 and KLM-1 cells formed spheroids in NanoCulture plates, but PK-45H and MIAPaCa-2 did not. Between 2D and 3D cultures, distinct expressions of F-actin and tubulin were observed, and these differences might affect morphological changes of PDAC cells. E-cadherin might be one of the key molecules involved in spheroid formation of PDAC cells. Furthermore, the present study showed that the 3D spheroidal culture system was useful for cell imaging with contrast-phase microscopy and confocal laser scanning microscopy. The newly developed 3D spheroidal culture system might help translational research in cancer biology.

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References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008) Cancer statistics, 2008. CA Cancer J Clin 58:71–96
2. Bardeesy N, DePinho RA (2002) Pancreatic cancer biology and genetics. Nat Rev Cancer 2:897–909
3. Nelson CM, Bissell MJ (2006) Of extracellular matrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol 22:287–309
4. Yamada KM, Cukierman E (2007) Modeling tissue morphogenesis and cancer in 3D. Cell 130:601–610
5. Vincan E, Brabletz T, Faux MC, Ramsay RG (2007) A human three-dimensional cell line model allows the study of dynamic and reversible epithelial-mesenchymal and mesenchymal-epithelial transition that underpins colorectal carcinogenesis. Cells Tissues Organs 185:20–28
6. Schagemann JC, Mrosek EH, Landers R, Kurz H, Erggelet C (2006) Morphology and function of ovine articular cartilage chondrocytes in 3-D hydrogel culture. Cells Tissues Organs 182:89–97
7. Sakai T, Larsen M, Yamada KM (2003) Fibronectin requirement in branching morphogenesis. Nature (Lond) 423:876–881
8. Padron JM, van der Wilt CL, Smid K, Smitskamp-Wilms E, Backus HH, Pizao PE, Giaccone G, Peters GJ (2000) The multilayered postconfluent cell culture as a model for drug screening. Crit Rev Oncol Hematol 36:141–157
9. Yamada M, Moritoh C, Kawaguchi M, Okigaki T (1989) Growth, morphology, function, and morphogenetic properties of rat renal glomerular epithelial cells in vitro: effects of retinyl acetate. Eur J Cell Biol 49:252–258
10. Smalley KS, Lioni M, Herlyn M (2006) Life isn't flat: taking cancer biology to the next dimension. In Vitro Cell Dev Biol Anim 42:242–247
11. Ghosh S, Spagnoli GC, Martin I, Ploegert S, Demougin P, Heberer M, Reschner A (2005) Three-dimensional culture of melanoma cells profoundly affects gene expression profile: a high density oligonucleotide array study, J Cell Physiol 204:522–531
12. Shimada M, Yamashita Y, Tanaka S, Shirabe K, Nakazawa K, Ijima H, Sakiyama R, Fukuda J, Funatsu K, Sugimachi K (2007) Characteristic gene expression induced by polyurethane foam/spheroid culture of hepatoma cell line, Hep G2, as a promising cell source for bioartificial liver. Hepatogastroenterology 54:814–820
13. Takagi A, Watanabe M, Ishii Y, Morita J, Hirokawa Y, Matsuzaki T, Shiraishi T (2007) Three-dimensional cellular spheroid formation provides human prostate tumor cells with tissue-like features. Anticancer Res 27:45–53
14. Richert L, Binda D, Hamilton G, Viollon-Abadie C, Alexandre E, Bigot-Lasserre D, Bars R, Coassolo P, LeCluyse E (2002) Evaluation of the effect of culture configuration on morphology, survival time, antioxidant status and metabolic capacities of cultured rat hepatocytes. Toxicol In Vitro 16:89–99
15. Griffith LG, Swartz MA (2006) Capturing complex 3D tissue physiology in vitro. Nat Rev Mol Cell Biol 7:211–224
16. Marrero B, Messina JL, Heller R (2009) Generation of a tumor spheroid in a microgravity environment as a 3D model of melanoma. In Vitro Cell Dev Biol Anim 45:523–534
17. Kim JB (2005) Three-dimensional tissue culture models in cancer biology. Semin Cancer Biol 15:365–377
18. Kueh HY, Mitchison TJ (2009) Structural plasticity in actin and tubulin polymer dynamics. Science 325:960–963
19. Albiges-Rizo C, Destaing O, Fourcade B, Planus E, Block MR (2009) Actin machinery and mechanosensitivity in invadopodia, podosomes and focal adhesions. J Cell Sci 122:3037–3049
20. Small JV, Stradal T, Vignal E, Rottner K (2002) The lamellipodium: where motility begins. Trends Cell Biol 12:112–120
21. Kumar HR, Zhong X, Hoelz DJ, Rescorla FJ, Hickey RJ, Malkas LH, Sandoval JA (2008) Three-dimensional neuroblastoma cell culture: proteomic analysis between monolayer and multicellular tumor spheroids, Pediatr Surg Int 24:1229–1234