In Vivo Observation of Endothelial Cell-Assisted Vascularization in Pancreatic Cancer Xenograft Engineering

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Received: 14 November 2017 / Revised: 10 December 2017 / Accepted: 2 January 2018 / Published online: 3 February 2018

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Abstract In this study, for better understanding of patient-derived xenograft (PDX) generation, angiogenic characteristics during PDX cancerous tissue generation was investigated with different initial cell seeding conditions in the hydrogel. We monitored the angiogenic changes during the formation of *in vivo* cancer cell line xenografts induced by endothelial cells. Our *in vivo* cancer tissue formation system was designed with the assistance of tissue engineering technology to mimic patient-derived xenograft formation. Endothelial cells and MIA PaCa-2 pancreatic carcinoma cells were encapsulated in fibrin gel at different mixing configurations and subcutaneously implanted into nude mice. To investigate the effect of the initial cancerous cell distribution in the fibrin gel, MIA PaCa-2 cells were encapsulated as a homogeneous cell distribution or as a cell aggregate, with endothelial cells homogeneously distributed in the fibrin gel. Histological observation of the explanted tissues after different implantation periods revealed three different stages: isolated vascular tubes, leaky blood vessels, and mature cancerous tissue formation. The *in vivo* engineered cancerous tissues had leaky blood vessels with low expression of the vascular tight junction marker CD31. Under our experimental conditions, complex cancer-like tissue formation was most successful when tumorous cells and endothelial cells were homogeneously mixed in the fibrin gel. The present study implies that tumorous xenograft tissue formation can be achieved with a low number of initial cells and that effective vascularization conditions can be attained with a limited volume of patient-derived cancer tissue. Endothelial cell-assisted vascularization can be a potent choice for the effective development of vascularized cancerous tissues for studying patient-derived xenografts, cancer angiogenesis, cancer metastasis, and anticancer drugs.

Keywords Cancer tissue engineering · Vascularization · Patient-derived xenograft · Pancreatic cancer

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1 Introduction

Tissue maturation in an *in vivo* environment using cells and scaffolds is a basic concept in tissue engineering and regenerative medicine [1, 2]. A typical tissue engineering process involves the proliferation of cells from a variety of cell sources, cell seeding on a three-dimensional scaffold, *in vitro* tissue maturation, and ultimate reconstitution or regeneration in the target site of the patient via transplantation [2]. This tissue engineering concept has been extensively studied for almost all types of tissues, including the myocardium, nerves, lung, liver, skin, blood vessels, skeletal muscle, bone, cornea, and cartilage [3–9].

In recent years, this concept has been applied to the *in vivo* discovery of therapeutic drugs using cancer tissues [10–12]. Briefly, primary tumor cells from a patient are injected into the body of immunocompromised mice and then vascularized to form a tumor tissue mass. The resulting cancerous tissues have similar pharmacodynamic response characteristics to the original tumor tissue, and researchers can determine treatment strategies for patients with the particular type of cancer. Tumor tissue animal models have been extensively studied for patient-specific drug screening. Patient-derived xenograft cancerous tissue (PDX) helps in the selection of drugs for the therapeutic treatment of cancer patients by allowing researchers to determine the genetic profiles of cancerous tissues in patients at different maturation stages. Additionally, growth of tumor tissue in an animal's body sheds light on various cancer biology aspects, such as cancer development, growth, metastasis, and anticancer drug screening [13–15].

In contrast to conventional two-dimensional cancer cell culture models, cancer tissue grown *in vivo* can reflect the biological characteristics of a particular cancer tissue. Furthermore, the development of cancerous tissues in the body can provide valuable information on mechanisms, factors important for tissue formation, and various current cancer research approaches [16]. The PDX model is believed to more closely represent the status of human cancer than the two-dimensional cell culture approach when determining if a patient's tumor would respond to a specific treatment regimen. However, the generation of PDX tumor tissue in the mouse model is still limited. For example, the number of cells implanted in mice is not optimized and some types of tumor cells do not proliferate to form tumor tissue [16–18]. Additionally, it would be beneficial if there is a way to generate PDX using fewer cells, enabling PDX to be developed from early cancer tissue biopsies for therapeutic drug screening and possibly improve patients' therapeutic outcomes [19]. Finally, the screening of anticancer drugs by the PDX animal model requires improvement due to the limited success of PDX formation.

In our current study, we hypothesized that early angiogenesis would boost PDX formation from a low number of cells. As an angiogenic strategy, endothelial cells encapsulated in fibrin hydrogels, which has been shown having angiogenic and vasculogenic characteristic [20, 21]. Fibrin gels are often employed as an angiogenic matrix *in vitro* and *in vivo* [22]. Mia PaCa-2, a human pancreatic adenocarcinoma cell line, was used as a model cancer cell. Cancer and endothelial cell lines embedded in fibrin gels were implanted *in vivo* and the occurrence of xenografts was observed for up to 8 weeks. Angiogenic xenotransplantation techniques using cancer cell lines can reveal the biological characteristics of various types of cancer tissues

and may suggest useful tools for determining regimens for anticancer treatment administration.

2 Materials and methods

2.1 Materials

Bovine fibrinogen, thrombin, and aprotinin from bovine lung were purchased from Sigma-Aldrich (Sigma-Aldrich Korea, Yongin-si, Gyeonggi-do, Korea). Human thrombin was obtained from Reyon Pharmaceutical Co., Ltd. (Seoul, Korea). Phosphate-buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and antibiotic–antimycotic solution (AA) were purchased from Life Technologies Korea (Seoul, Korea). Anti-von Willebrand Factor (vWF) was from Santa Cruz (Dallas, TX), Anti-CD31 and anti- α -SMA was from Abcam and polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning Korea (Seoul, Korea). MS1 (mouse pancreatic islet endothelial cell line) and MIA PaCa-2 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA).

2.2 Preparation of fibrin gel

Fibrinogen solution was prepared by dissolving fibrinogen in PBS to a 50 mg/ml concentration at 37 °C for 2 h, and thrombin solution was prepared by dissolving 100 IU/ml thrombin and 200 μ g/ml aprotinin in PBS. Each solution was sterilized with 0.22- μ m pore-size syringe filters prior to use. Disc-shaped fibrin samples were fabricated by casting a mixture of the fibrinogen and thrombin solutions in PDMS wells (diameter 8 mm; thickness 5 mm). During mixing, cell culture medium with 2×10^5 cells was added to the fibrinogen solution (1:1 volume ratio). Fibrinogen and thrombin solution was mixed at a 4:1 volume ratio, resulting in final concentrations of 20 mg/ml fibrinogen, 20 IU/ml thrombin, and 40 μ g/ml aprotinin. A 200 μ l volume of each premixed solution was transferred to each PDMS well and gelled.

2.3 Cancer cell-embedded hydrogel preparation

Four experimental groups were prepared according to embedded cell and sample conditions ($n = 3/\text{group}$): (1) control with no cells in the fibrin (CON), (2) endothelial MS1 cells (2×10^5 cells per sample) homogeneously distributed in the fibrin (EH), (3) endothelial MS1 cells (2×10^5 cells) homogeneously distributed with the cancer aggregate (1×10^5 MIA PaCa-2 cells) located in the center of the fibrin [MS1:MIA PaCa-2 (aggregate) = 2:1] (EHCA), (4) endothelial MS1 cells (2×10^5 cells) and

cancer cells (1×10^5 MIA PaCa-2 cells) homogeneously distributed in the fibrin (MS1:MIA PaCa-2 = 2:1) (EHCH). After gelling for 30 min at room temperature, each hydrogel sample was transferred to containers with cell culture medium for *in vivo* study. The hydrogel samples were cultured in high-glucose DMEM containing 5% FBS and 1% AA and incubated at 37 °C in a humidified incubator with 5% CO₂ for 1 day.

2.4 *In vivo* cancer tissue engineering experiment

Animal research protocols were peer reviewed and approved by the Asan Medical Center IACUC (Protocol Number: 2014-12-003). All animal procedures were conducted in accordance with the declaration of the World Medical Association of Helsinki [23]. Fibrin samples were transplanted into the dorsal subcutaneous pocket of athymic nude mice (8 weeks old; Orient Bio Inc., Seongnam, Korea) (Fig. 1). At 1, 2, 4, and 8 weeks after implantation, the samples were removed and fixed with 4% paraformaldehyde for further characterization. Fibrin gel samples were implanted into four animals in each group. Thus, a total of 16 animals underwent the *in vivo* experiments.

2.5 Histological analysis

The harvested samples were fixed in 4% paraformaldehyde and embedded in OCT compound (Sakura, Torrance, CA). The embedded samples were frozen and sectioned at 20 µm thickness with a cryotome and stained using Mayer's hematoxylin and eosin solution (Sigma-Aldrich Korea).

Fig. 1 Schematic diagram of the pancreatic cancer xenograft engineering. Four experimental groups were prepared according to embedded cell and sample conditions: (1) control with no cells in the fibrin (CON), (2) endothelial cells homogeneously distributed in the fibrin (EH), (3) endothelial cells homogeneously distributed with the cancer aggregate located in the center of the fibrin (EHCA), (4) endothelial cells and cancer cells homogeneously distributed in the fibrin (EHCH)

2.6 Immunofluorescence staining

Samples were immunolabeled for vWF, CD31 and α -SMA expression. In brief, frozen sections on slides were permeabilized with 0.1% Triton X-100 in PBS for 30 min, washed with PBS for 5 min, blocked with 3% human serum albumin in PBS for 1 h before antibody binding, and then incubated with anti-vWF (1:100), anti-CD31 (1:100) and anti- α -SMA (1:100) solution for 1 h. Finally, the samples were incubated with secondary antibody for another 1 h, washed three times with PBS, and mounted with Prolong Gold containing 4',6-diamidino-2'-phenylindole dihydrochloride (DAPI).

2.7 Fluorescence and confocal microscopy

vWF-immunolabeled tissue slides were observed with fluorescence microscopy (EVOS FL Auto; Life Technologies Korea) at an excitation wavelength of 350 nm and emission wavelength of 488 nm; DAPI was observed with a 400-nm filter. Fluorescence images of CD31 and α -SMA were acquired with a confocal microscope (Zeiss Axio Vert). During fluorescence imaging, the exposure time and signal amplification ratio were fixed for further image analysis. For imaging, one cryosectioned tissue sample from each animal was used ($n = 4$ for each time point and each condition), and at least three images were taken from each tissue slide.

2.8 Image analysis of vascularization

Fluorescence images of vWF, CD31 and α -SMA were analyzed with ImageJ (<https://imagej.nih.gov/ij/download.html>) according to previous reports [24]. Briefly, from each image, RGB channels were separated and the area fraction for each color was measured. At least three area fractions were measured from each image.

2.9 Statistical analysis

Results are expressed as mean $\pm$ SE. Comparisons were made using the Student's *t* test. Differences were considered significant at $p < 0.05$.

3 Results and discussion

In our present study, endothelial cells were used to support vascularization and cancer tissue formation. Additionally, adenocarcinoma cells were embedded as single cells or a cell aggregate to identify the optimal initial conditions for effective xenograft tissue formation. The tissue and blood vessel formation capabilities of MIA PaCa-2 pancreatic adenocarcinoma cells differed according to the initial cell distribution.

3.1 Gross observation of *in vivo* tissue explants

The gross images of the fibrin gel explants after predetermined periods are shown in Fig. 2. Under visual observation, the redness of the explants was used to reflect the degree of blood supply from the host circulatory system. In the CON group samples, there was no evidence of blood supply or tissue formation and the fibrin gel implants showed negligible changes in color and transparency during the 8-week period (Fig. 2A, E, I, M). In contrast, explants of the endothelial cell-loaded groups—EH, EHCA, and EHCH—showed a gradual change in redness, implying a progressive increase in blood supply and vascularization within the implantation period. In the EH group, explants started to turn red from week 2 (Fig. 2F, J, N). The EHCA group explants showed active blood supply in the 2-week samples. Compared with the EH group explants, the red color appeared to be darker in the EHCA group 2 weeks after implantation, but there was no significant difference between the two groups 8 weeks after implantation (Fig. 2F, G). In the EHCA group, the size of the explanted sample gradually decreased and was the smallest of all groups at the 8-week implantation stage (Fig. 2O). The EHCH group started to show redness in 2-week explants, presumably due to the host blood supply. The overall size of the EHCH group decreased during the first 4 weeks but was

increased 8 weeks after transplantation (Fig. 2D, H, L, P). Interestingly, turbid regions appeared in the 4-week samples and the region was increased in size in the 8-week samples. At 4 and 8 weeks, the color of the turbid region of the EHCH group was partly cloudy and significantly different from that of the other three groups. This semitransparent tissue region appeared in the 4-week samples and occupied most of the 8-week samples.

Optical microscope observation of the explants gave a closer look at the blood vessel formation. Figure 3 shows the optical microscope images of the 1-week and 4-week *in vivo* samples. After 1 week, a significant blood supply was only observed in the EHCA group (Fig. 3A–D). However, after 4 weeks, a blood vessel network was observed in all endothelial cell-containing conditions, whereas control fibrin samples showed no blood vessels in the hydrogel (Fig. 3E–H). An initial stage of blood perfusion was observed in the 1-week EHCA samples, which was identified by localized red-colored vessel perfusion. These early vascular networks (shown in Fig. 3C) explain the higher host blood perfusion of the 2-week EHCA explants (Fig. 2C).

3.2 Histological observation

Optical microscopy of micro-sectioned and hematoxylin and eosin-stained specimens are shown in Figs. 4 and 5. In the CON group, cells were localized along the outer boundary at both 4 weeks and 8 weeks and few cells were observed in the central region until 8 weeks after implantation (Figs. 4H, L, 5H, L). In the EH, EHCA, and EHCH groups, HUVECs were initially mixed in the fibrin gel implants and these endothelial cells interconnected to form endothelial tissue after 1 week (Fig. 5B–D). After 2 weeks, all of the HUVEC mixed groups showed red blood cells in the middle of the specimen, implying that tubular blood vessels formed and connected to the host circulation system (Fig. 5E, F, J). In the EHCA group, the inserted cell aggregates remained in the center region of the implants with a high cell population up to 8 weeks. However, this high cell population region did not expand until 8 weeks after implantation (Fig. 4N). In the EHCH group, the cell concentration in the fibrin gel had increased to a very high level by 4 and 8 weeks. Most of the volume of the fibrin gel was filled with cells at a high population density and the fibrin gel matrices were almost disappeared 8 weeks after implantation in the EHCH group (Figs. 4O, 5O).

There were significant differences in tissue formation in the 8-week explants. The CON group remained non-vascularized and only a small number of cells were observed near the center of the implanted fibrin gel (Figs. 4L, 5L). The fibrin gel remained at the center in the EH group, but the boundary region was filled with cells and blood vessels (Figs. 4M, 5M). In the EHCA group, a high cell-density tissue region formed

Fig. 2 Gross observation of *in vivo* tissue explants. At 1, 2, 4, and 8 weeks of after implantation, the samples were removed and observed. **A, E, I, M** Control group, **B, F, J, N** EH group, **C, G, K, O** EHCA group and **D, H, L, P** EHCH group

Fig. 3 Optical microscope observation of the harvested samples after 1 and 4-week implantation. **A, E** Control group, **B, F** EH group, **C, G** EHCA group and **D, H** EHCH group. Scale bar = 200 μ m

during the first week, which promoted blood vessel formation (Fig. 3C), but inhomogeneous vascularization and cell proliferation was observed after 8 weeks in the remaining fibrin

gel matrix (Figs. 4N, 5N). In the EHCH group, fibrin gels were negligibly observed after 8 weeks as the explants were fully filled with cells. Furthermore, an increased tissue size

Fig. 4 Gross observation of *in vivo* implanted fibrin gel after H&E staining. Images are adjusted to same scale for visual comparison of samples size. **A, H, L** Control group, **B, E, I, M** EH group, **C, F, J, N** EHCA group and **D, G, K, O** EHCH group. Scale bar = 500 μ m

was observed when 4-week and 8-week implantation samples were compared, reflecting the formation and gradual growth of tumorous tissues.

These results indicated that the initial carcinoma cell mixing condition affects xenograft tissue formation. When a carcinoma cell aggregate was mixed in the fibrin gel with MS1 cells, tumorous xenograft formation was slower than in fibrin gels with homogeneously distributed carcinoma cells (Fig. 4N, O). The EHCH group with carcinoma cells homogeneously mixed with MS1 cells had better xenograft tumorous tissue formation than the other three groups (Fig. 4L–O).

3.3 Vasculogenesis in the *in vivo* pancreatic cancer xenograft tissue model

Time-dependent angiogenesis was observed in all endothelial cells-containing groups using images of hematoxylin and eosin-stained and immunofluorescence-stained specimens. All endothelial cell-containing groups showed

elongated blood vessels 1 week after implantation. Most of the endothelial cells showed a short-range connection with neighboring cells and formed isolated linear structures (black arrows in Fig. 5B–D). However, there were differences in the length of the vascular structures in the explants, reflecting the effect of the initial carcinoma cell distribution. Figure 6 shows the microvasculature lengths of 1-week explants with the frequency in percentage at 50- μ m intervals and the mean $\pm$ SD length comparison. The microvasculature length of the EH, EHCA, and EHCH groups at week 1 were 106.2 ± 65.9 , 202.3 ± 128.5 , and 111.7 ± 59.8 μ m, respectively (Fig. 6D). In addition, the EHCA group showed a higher fraction of long blood vessels than the EH and EHCH groups ($p < 0.001$). The fractions of microvasculatures longer than 200 μ m in the EH, EHCA, and EHCH groups were 20.6, 45.2, and 22.7%, respectively (Fig. 6A–C and Table 1). In contrast, there were no significant differences in the mean value and fractional distribution of microvasculature length between the EH and EHCH groups in 1-week implanted specimens.

Fig. 5 Histological observation of *in vivo* implanted cell embedded fibrin gel. At 1, 2, 4, and 8 weeks of after implantation, the samples were H&E staining. **A, H, L** Control group, **B, E, I, M** EH group, **C,**

F, J, N EHCA group and **D, G, K, O** EHCH group. Scale bar = 200 μm . (Color figure online)

3.4 Immunofluorescence of vascularization markers

vWF, a vascular endothelial cell marker, was expressed in all MS1s mixed implants. Figure 7 shows fluorescence microscopy images of explants stained with human vWF antibody. Initially mixed endothelial cells were viable and supported vascularization during the pancreatic carcinoma xenograft formation. Figure 8 shows immunofluorescence images of CD31 and alpha smooth muscle actin, which are markers of mature blood vessels. From the assessment of the area fraction of each marker, there was no significant difference in CD31 or α -SMA expression among the three groups. However, there was a tendency for increased CD31 and α -SMA with time in all groups. In particular, the EHCH group showed the highest increase in terms of the 8-week to 4-week ratio in the mean value of the area fraction of CD31-conjugated antibody (Fig. 8C, F, G). Additionally, the EHCH group showed a lower area fraction of smooth muscle actin than the other two groups. α -

SMA expression shows a mature vessel, can be a blood vessel wall forms a tight junction, which may means that tumor tissue is not developed. Compared with this, in the EHCH group, due to the development of tumor tissue, the leaky blood vessel wall developed and there was no mature blood vessel formation and α -SMA was not developed.

The formation of microenvironments such as the extracellular matrix and microvascular network is an important factor for cell survival and *in vivo* tissue formation [25]. Patient-derived cancer cells are usually obtained from a biopsy or excised cancer tissues, sometimes from less than 5 mm³ tumor tissues remaining after pathological assessment [15, 26]. With a small number of explanted cancer cells, it is important to regulate the microenvironment of the transplanted cells for successful *in vivo* cancer xenograft formation. Patient-derived xenograft models are typically investigated using immunodeficient rodents implanted with tumorous biopsies or cultured cancer cells [17, 27, 28]. For different tumor types,

Fig. 6 Blood vessel length comparison after 1-week implantation. **A** EH group, **B** EHCA group, **C** EHCH group and **D** cross-group comparison graph

Table 1 Microvasculature length and length fraction

Group	EH	EHCA	EHCH
Mean	106.2499	202.332	111.6663
SD	65.87202	128.5286	59.78364
Longer than 200 μm	2.6	45.2	22.7

particularly the brain and hormone-dependent tumors, the variability in the PDX success rate is an issue that remains to be resolved [29–31]. Pancreatic cancer xenografts generated with MIA PaCa-2 and MS1 cells can be a potent model system for effective pancreatic ductal

adenocarcinoma PDX formation [32, 33]. The *in vivo* results suggest that endothelial cells should be mixed with an appropriate hydrogel as an extracellular matrix, such as fibrin, to boost the angiogenesis of the new cancer tissues [34].

In our present study, because the MIA PaCa-2 cell line has colonizing capacity, the cells proliferated and formed colonies or large aggregates in the fibrin matrix after 1 week *in vivo* in both the EHCH and EHCA groups. During metastasis, tumor cells penetrate through the blood or lymphatic vessels, circulate and move to new sites, and proliferate. Above a certain size, without appropriate blood vessel connections, cancer cells cannot proliferate, leading

Fig. 7 Immunofluorescence image of vascular marker von Wilbrand factor (vWF, green) expression in *in vivo* implanted fibrin gels for 4- and 8-week. All endothelial cell mixed explants were stained with vWF implementing blood vessel formation. **A, D** EH group, **B, E** EHCA group and **C, F** EHCH group. Scale bar = 100 μm. (Color figure online)

Fig. 8 Vasculogenesis in the *in vivo* pancreatic cancer xenograft tissue model. Immunofluorescence image of CD31 (green) and α -SMA (red) expression in *in vivo* implanted fibrin gels for 4- and

8-week. **A, D** EH group, **B, E** EHCA group and **C, F** EHCH group. Scale bar = 50 μ m. **G** Area fraction of alpha smooth muscle actin and CD31 expression. (Color figure online)

to necrotic tissue collapse and retarded cancer growth [35]. The *in vivo* results of the EH, EHCA, and EHCH groups showed that blood vessels did form but were not connected to the cancer aggregates until 1 week after implantation. However, carcinoma cell aggregates mixed prior to implantation in the EHCA group did not show effective cancerous tissue growth compared with the homogeneous mixture of MS1 cells and carcinoma cells in the EHCH group (Fig. 4). This result can be explained by the high oxygen demand of adenocarcinoma cell aggregates in the EHCA group before connection to the host circulation system, which caused a necrotic environment in the implants. Spheroids can grow to a diameter of about 1 mm size, and necrosis and hypoxia occur in the center as they grow larger [36]. In the group of EHCA, the size of aggregate is about 1 mm and the diameter of fibrin gel is about 6 mm, it is considered that similar necrosis occurred. In contrast, the EHCH group showed homogeneous tissue formation, with the adenocarcinoma cells presumably surviving the early necrotic period due to less severe oxygen deficiency because of the dispersed cancer cell colonies. These results indicate that early connection to the host circulation system is essential for cell survival and cancerous xenograft tissue growth [37, 38]. Furthermore, success in xenograft engineering also depends on limited oxygen deficiency in first implant stages. In addition to an angiogenesis microenvironment, a long-lasting microvascular structure can be achieved by the addition of perivascular cells that assist stable capillaries, with blood

vessels reported to be functional for more than a year [39–41].

3.5 Blood vessel maturation stages in engineered cancer tissues

We observed from our present analyses that there are three different stages in blood vessel maturation during pancreatic adenocarcinoma xenograft engineering. In the first stage (week 1), endothelial cells connect to form blood vessel-like tubular structures (Fig. 5B–D). At this stage, endothelial microvessels are not connected to the host circulation system. In the next stage, microvessels are connected to the host circulation system and supplied with blood but have leaky vessel walls. Thus, the blood vessels are not fully developed and red blood cells can leak out of the blood vessels. Large volumes of red blood cell clumps were observed in histological images of the explanted samples after 2 weeks (Fig. 5E, F; blue arrows). After the second stage, new tissue and blood vessels are fully matured and stable and no further red blood cell leakage occurs; this is the final step of cancerous tissue generation. In our current study, CD31, a marker of mature blood vessels, was not highly stained nor smooth muscle actin was highly expressed at this stage. The growth of mature functional tissues with tissue- or organ-specific ultrastructure needs to be explored in both *in vitro* and *in vivo* environments (Fig. 8).

3.6 Cancer tissue engineering applications

Based on our present findings, when endothelial cells are injected into xenograft tumor cells, a microvascular structure forms within a short time, supporting cancer cell survival and boosting the successful production of xenogeneic cancer tissue. Xenotransplantation of human-derived cancer cells or tumor cells into immunocompromised animals is a useful tool for preclinical screening of cancer therapies. Cancer cell lines can also produce cancerous tissue, but the resulting xenografts have failed to accurately predict clinical drug responses [42]. PDX models have shown that the gene expressions, drug responses, and the histology of PDX cancer tissues are generally highly stable and consistent with those of the original tumor samples [17, 43].

Xenotransplantation in immunodeficient rodents during cancer cell transplantation from patients has not always been successful. Solid tumor cells have shown a high success rate but cell hormone-dependent organs showed limited success [44, 45]. Some types of cancer cells are not effective at forming xenotransplants, and the help of a microvascularized environment may enhance early cell survival and cancer tissue formation and maturation.

In conclusion, we have demonstrated *in vivo* 3D cancer xenograft tissue formation with the use of fibrin hydrogel as a scaffold material and an endothelial cell line as an assisting component for vasculogenesis. Vascularization was found to be enhanced, presumably due to the presence of the endothelial cells. Cancer xenograft tissue formation was also boosted by homogeneous mixing of adenocarcinoma cells with endothelial cells. These results may help to improve the outcomes of PDX development for patients and support specific drug screening and therapeutic approaches in the future.

Funding This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (Grant Number: NRF-2015K1A4A3046807), by the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant Number: HI14C2640) and by the ASAN Institute for Life Sciences, ASAN Medical Center, Seoul, Korea (Grant Numbers: 2013-7206, 2014-7201, 2015-7213).

Compliance with ethical standards

Conflict of interest There are no conflicts to declare.

Ethical statement The animal experiment protocol was reviewed and approved by the institutional animal care and use committee of Asan Medical Center (Protocol Number: 2014-12-003).

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