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A Universal Gelfoam 3-D Histoculture Method to Establish Patient-derived Cancer Cells (3D-PDCC) Without Fibroblasts from Patient-derived Xenografts

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Abstract. *Background/Aim: The direct placement of patient tumors in 2-D culture on plastic or glass surfaces has inhibited the establishment of patient-derived cancer cells (PDCCs). The aim of the present study was to develop universal and efficient methods to prepare PDCCs. Materials and Methods: Fragments of patient-derived xenograft (PDX) tumors established form colon cancer liver metastasis (1 mm^3) were placed on Gelfoam and cultured in DMEM. Results: PDX tumor fragments were cultured on Gelfoam. Cancer cells migrated from the explant and formed distinct 3-D structures in the Gelfoam. Each of the three PDCCs showed a distinct morphology. The cultures were essentially all cancer cells without fibroblasts, the opposite of what usually occurs in 2-D culture on plastic or glass. Gelfoam cultures could be readily passaged from one Gelfoam cube to anothers suggesting indefinite culture potential. Conclusion: A potentially universal method to establish PDCC using PDX tumors and 3-D Gelfoam histoculture was developed.*

Each cancer patient's tumor contains an enormous wealth of information, that if properly extracted, can be used to design improved treatment for the patient as well as help in understanding the basic biology of cancer. Cancer cell lines have been established from patient tumors over the past 75 years with limited success (1). There are two important reasons why most attempts to establish patient-derived cancer cell lines (PDCCs) have failed: 1) The attempts to culture the cancer cells have been made on plastic or glass surfaces, highly artificial environments for cancer cells and, 2) The tumors used have not been established to grow outside the body.

Sponge-matrix 3-D culture was developed by Leighton 70 years ago (2, 3). Sponge-matrix culture enables cancers, including their native stroma to grow, forming 3-dimensional *in vivo*-like structures, including glioma (2-4). Our laboratory has shown that all types of cancer can grow on Gelfoam sponge and migrate throughout the gel. We have also shown that Gelfoam histoculture is genuine 3-D as compared to Matrigel, which is at best 2.5-D (5).

In 1969, Rygaard showed that patient tumors could be established in nude mice for unlimited passaging (6).

In the present report, we took advantage of the patient-derived tumor established to grow in mice (patient-derived xenografts, PDX), and used the established tumors for culture on Gelfoam to make PDCC, thereby avoiding the use of primary unestablished tumors and 2-D culture on plastic, which selects against the growth of most cancer cells.

Materials and Methods

Animal studies. Athymic *nu/nu* mice (AntiCancer Inc, San Diego, CA, USA), 4-6 weeks old, were used in this study. All mice were kept in a barrier facility on a high efficacy particulate air (HEPA)-filtered rack under standard conditions of 12 h light/dark cycles. Animal studies were performed with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specially

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Key Words: 3-D culture, histoculture, Gelfoam, patient-derived cancer cells, PDCC, patient-derived xenograft, PDX.

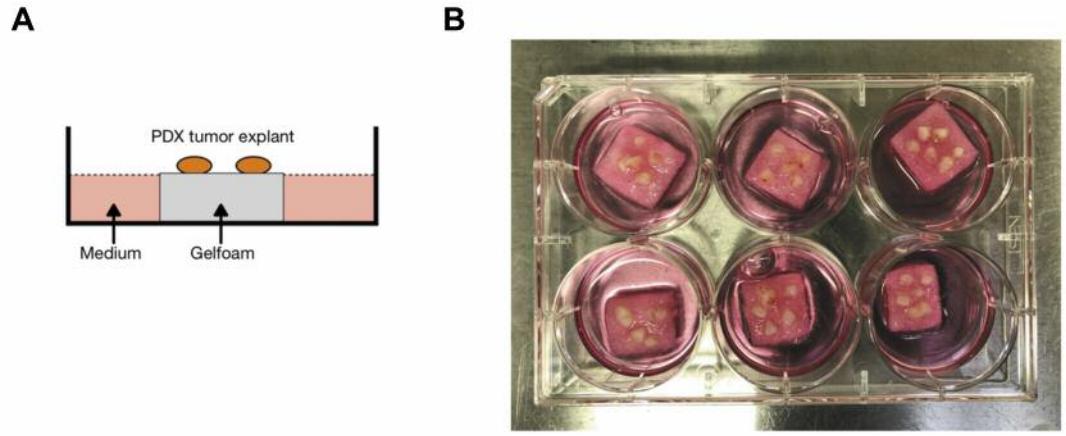

Figure 1. *Start of 3-D Gelfoam histoculture of tumor fragments from a patient-derived xenograft (PDX) colon-cancer liver metastasis. (A) Diagram of 3-D Gelfoam tumor histoculture. (B) Photograph of 3-D Gelfoam histocultures with fragments of a colon-cancer liver metastasis PDX.*

approved for this study, in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

Patient tumors. Three different patients with liver metastasis of colon cancer (CM1, CM2 and CM3, respectively) underwent liver resection in the Department of Surgery, University of California, San Diego (UCSD), USA, under IRB protocol 140046 with patients' informed consent (7). A small tumor sample was obtained from each patient at the time of the surgical operation and immediately transported to the laboratory on ice. The fresh specimens were implanted into a subcutaneous pocket of nude mice to establish their growth (8).

Gelfoam 3-D histoculture. Sterile Gelfoam sponges (Pharmacia & Upjohn, Kalamazoo, MI, USA), prepared from porcine skin, were cut into 1 cm cubes. The Gelfoam cubes were placed in six-well tissue-culture plates. Dulbecco's modified Eagle's medium (DMEM) was added until the upper part of Gelfoam was reached but not covered (Figure 1A), and the plates were incubated at 37°C in order for the Gelfoam to absorb the medium. The tumor fragments were placed on the top of the Gelfoam (Figure 1B).

Histological examination. Fixation, paraffin-embedding of tumor fragments along with Gelfoam and deparaffinization and rehydration procedures were performed as previously described (9). Hematoxylin and eosin (H&E) staining was performed using a standard protocol.

Results

Three patient colon cancer liver metastases, CM1, CM2, CM3 were established as patient-derived xenografts in nude mice as previously described (8). CM1 tumor fragments (1 mm^3) were placed on the Gelfoam cubes in medium (Figure 2A). At that time, numerous cancer cells grew from the explants and formed the complex structures of an adenocarcinoma on and within the Gelfoam (Figure 2B). Numerous cancer cells were growing out of the tumor fragments of the on the Gelfoam (Figure 2C). Gelfoam begins to dissolve after long-term culture. The dissolving pieces of the Gelfoam were placed on fresh Gelfoam in culture medium (Figure 3A and B) and the cancer cells migrated to the new Gelfoam and grew extensively without fibroblasts (Figure 3C). Thus, Gelfoam cultures can be easily passaged and expanded. It should be noted the PDCCs can form complex 3-D structures on the Gelfoam (Figure 3B).

Discussion

Few cancer cell lines have been established over the past 75 years due to the fact that unestablished tumors were used as a cell source and plastic and glass was used as the surface for the cancer cell growth, thus, precluding the development of cell lines from the majority of cancers (1). PDX establishment and 3-D Gelfoam histoculture have solved these problems. We have recently developed a new technique to greatly improve the rate of patient tumor establishment in nude mice (8). With the high frequency of PDX establishment, and the fact that most PDX-tumors can produce PDCC on Gelfoam, the production of PDCCs from most patients' tumors has become possible. The PDCCs in 3-D Gelfoam culture can be used to test drug sensitivity using the histoculture drug-sensitivity assay (HDRA), which has been validated in many clinical trials to correlate with patient response (10-12). PDCCs can also be used to study basic cancer biology. In conclusion, the present study shows that PDCCs can be established from PDX tumors using 3-D Gelfoam histoculture, without fibroblasts. We expect that PDCCs can be established following surgery or biopsy from the majority of cancer patients. The PDCCs can be used for the patients' benefit and for the benefit of cancer research.

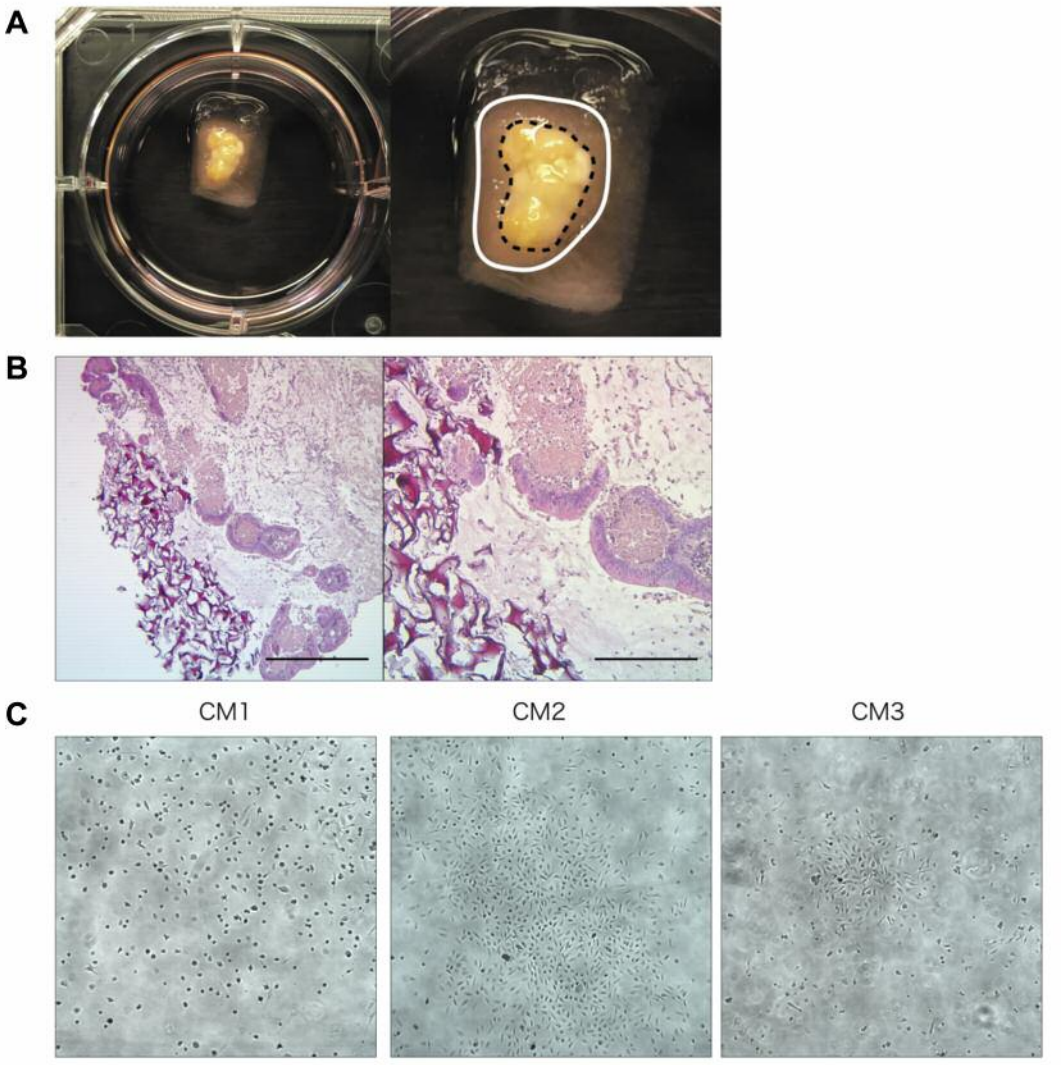

Figure 2. *Long-term 3-D Gelfoam histoculture of CM1, CM2 and CM3. (A) Histoculture of CM3 for 8 weeks. Left: macrophoto 3-D Gelfoam histoculture. Right: Close-up photo. Black black broken line: growing tumor explant; white line: numerous cancer cells invading into the Gelfoam. (B) Images of H & E staining of CM1. Left: ×40, Black bar, 500 μm. Right: ×100 Black bar, 200 μm. Note the complex 3-D structures formed by the PDCC. (C) Morphologies of the PDCC of CM1, CM2 and CM3. Note the absence of fibroblasts. Magnification: ×100.*

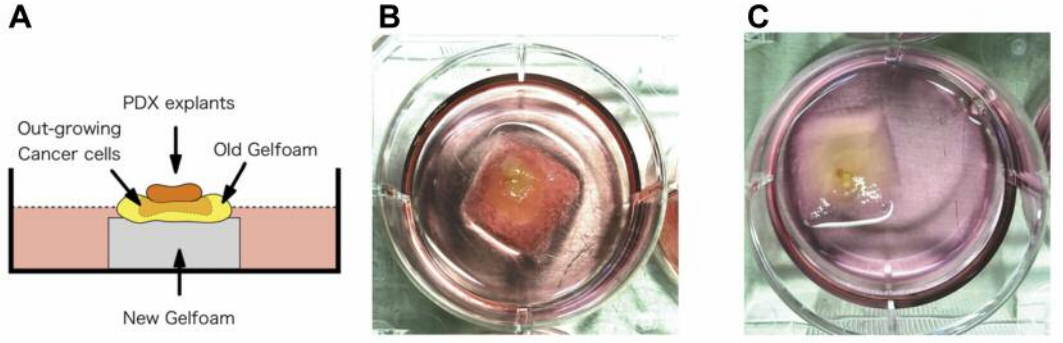

Figure 3. *Passage from old to new 3-D Gelfoam histocultures. (A) Diagram of passage from old to new 3-D Gelfoam culture. (B) Immediately after patient-derived cancer cell passage on fresh Gelfoam. (C) Four weeks after passage; numerous PDCC are growing on the new Gelfoam cube.*

Conflicts of Interest

JY, QH, SI, NS, KH, HN and RMH are or were unsalaried associates of AntiCancer Inc. The Authors declare that there are no potential conflicts of interest regarding this study.

Authors' Contributions

J.Y. and R.M.H designed and performed experiments and wrote the paper; N.S., K.H., N.H., K.M., R.M., S.I., H.T. and M.B. gave technical support and conceptual advice. Writing, review, and/or revision of the manuscript: J.Y., I.E. and R.M.H.

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References

1 Hoffman RM: Tissue culture. *In*: Brenner's encyclopedia of genetics (second edition). Maloy S and Hughes K (eds.). Academic Press: San Diego, pp. 73-76, 2013. DOI: 10.1016/B978-0-12-374984-0.01543-6

2 Leighton J: A sponge matrix method for tissue culture; formation of organized aggregates of cells *in vitro*. J Natl Cancer Inst *12(3)*: 545-561, 1951. PMID: 14889259.

3 Leighton J: The growth patterns of some transplantable animal tumors in sponge matrix tissue culture. J Natl Cancer Inst *15(2)*: 275-293, 1954. PMID: 13233883.

4 Freeman AE and Hoffman RM: *In vivo*-like growth of human tumors *in vitro*. Proc Natl Acad Sci USA *83(8)*: 2694-2698, 1986. PMID: 3458228. DOI: 10.1073/pnas.83.8.2694

5 Tome Y, Uehara F, Mii S, Yano S, Zhang L, Sugimoto N, Maehara H, Bouvet M, Tsuchiya H, Kanaya F and Hoffman RM: 3-dimensional tissue is formed from cancer cells *in vitro* on Gelfoam®, but not on matrigel™ . J Cell Biochem *115(8)*: 1362-1367, 2014. PMID: 24497277. DOI: 10.1002/jcb.24780

6 Rygaard J and Povlsen CO: Heterotransplantation of a human malignant tumour to "nude" mice. Acta Pathol Microbiol Scand *77(4)*: 758-760, 1969. PMID: 5383844. DOI: 10.1111/j.1699-0463.1969.tb04520.x

7 Hollandsworth HM, Amirfakhri S, Filemoni F, Schmitt V, Wennemuth G, Schmidt A, Hoffman RM, Singer BB and Bouvet M: Anti-carcinoembryonic antigen-related cell adhesion molecule antibody for fluorescence visualization of primary colon cancer and metastases in patient-derived orthotopic xenograft mouse models. Oncotarget *11(4)*: 429-439, 2020. PMID: 32064046. DOI: 10.18632/oncotarget.27446

8 Murata T, Hozumi C, Hiroshima Y, Shimoya K, Hongo A, Inubushi S, Tanino H, and Hoffman RM: Co-implantation of tumor and extensive surrounding tissue improved the establishment rate of surgical specimens of human-patient cancer in nude mice: toward the goal of universal individualized cancer therapy. In Vivo *34(6)*: 3241-3245, 2020. PMID: 33144429. DOI: 10.21873/invivo.12160

9 Yamamoto J, Murata T, Sugisawa N, Higuchi T, Tashiro Y, Nishino H, Inubushi S, Sun YU, Lim H, Miyake K, Shimoya K, Nomura T, Kurebayashi J, Tanino H, Hozumi C, Bouvet M, Singh SR, Endo I and Hoffman RM: Eribulin regresses a cisplatinum-resistant rare-type triple-negative matrix-producing breast carcinoma patient-derived orthotopic xenograft mouse model. Anticancer Res *40(5)*: 2475-2479, 2020. PMID: 32366391. DOI: 10.21873/anticanres.14217

10 Vescio RA, Redfern CH, Nelson TJ, Ugoretz S, Stern PH and Hoffman RM: *In vivo*-like drug responses of human tumors growing in three-dimensional gel-supported primary culture. Proc Natl Acad Sci USA *84(14)*: 5029-5033, 1987. PMID: 3474637. DOI: 10.1073/pnas.84.14.5029

11 Furukawa T, Kubota T and Hoffman RM: Clinical applications of the histoculture drug response assay. Clin Cancer Res *1(3)*: 305-311, 1995. PMID: 9815986.

12 Hoffman RM: 3D sponge-matrix histoculture. Hoffman RM (ed.). Humana Press: New York, NY, 2018. DOI: 10.1007/978-1-4939-7745-1

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