Investigation of three-dimensional (3D) culture model and an anti-tumor drug screening system using PDX

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Objective

The patient-derived xenograft (PDX) is a model generated by direct transplantation of human tumor tissues into immunodeficient mice. Although a PDX model is known to have high clinical predictability, an in vivo model of PDX requires high trial costs and is very time-consuming. In order to solve these problems, we aimed to develop an anti-tumor drug evaluation system in vitro using PDXs. Firstly, we evaluated whether the tumor cells derived from colorectal, gastric and non-small cell lung carcinoma PDXs can be cultured in three dimensions (3D). As a result, the PDX-derived tumor cells formed a spheroidal cell aggregate (spheroid), and cell proliferation was observed. Secondly, we investigated the optimal conditions of 3D culture of the cells that had been disassociated from PDX tumors and frozen in liquid nitrogen. Lastly, we evaluated the anti-tumor effects of therapeutic agents against 3D-cultured PDX tumor cells.

Summary in Japanese

PDX (Patient-Derived Xenograft) は患者のがん組織をマウスに移植したモデルである．細胞株を用いたモデルと比較して *in vivo* PDXモデルの臨床予測性は高いことが知られているが，そのモデルを用いた試験は高い費用や長い期間を要するという課題を抱えている．そこで *in vitro* PDXに対する抗がん剤の薬効評価系の構築を試みた．国立がん研究センターより入手した大腸がん，胃がん，肺がん患者由来のPDXをマウスに移植した．一定の大きさに達した時点で腫瘍を摘出し，PDX腫瘍を分散した．分散したがん細胞の一部を3次元で培養し，一部を凍結保存した．3種のPDX由来のがん細胞は3次元の細胞塊（スフェロイド）を形成し，細胞増殖が認められた．さらに凍結保存から起眠したPDX由来のがん細胞も3次元で培養できた．次に3次元培養したPDX由来のがん細胞に対する抗がん剤の感受性を評価した．
その結果，使用したPDXや抗がん剤に応じたデータが得られ，評価系を確立できた．

今回検討した *in vitro* PDXの評価系は，*in vivo* PDXの抗がん剤の薬効評価の予測に利用できる可能性がある．

Materials and Methods

PDX*1	Origin
CO-043-LSIM	Colorectal cancer
GA-007-LSIM	Gastric cancer
LU-031-LSIM	non-small cell lung carcinoma

Cell disassociation materials: Tumor Dissociation Kit and GentleMACS Octo Dissociation with Heaters (Miltenyi Biotec)

Medium: Stem Fit (Ajinomoto Healthy Supply Co., Inc) or DMEM/F-12*2, GlutaMAX™ supplement (Thermo Fisher Scientific K.K.)

Extracellular matrix: Matrigel growth factor reduced (Corning Incorporated)

3D culture plate: Prime Surface 96U plate (Sumitomo Bakelite Co.,Ltd.)

Culture condition: CO_2 incubator set at 37.0℃ and 5.0% CO_2 under a humidified condition

Cell preservation reagent: CellBanker 1 (Takara Bio Inc)

Evaluation of cell viability: CellTiter-Glo 3D Cell Viability Assay (Promega Corporation)

*1 PDXs were obtained from the National Cancer Center Research Institute.
*2 This reagent was applied only for seeding the primary cell derived from LU-031-LSIM.

IC_{50} was calculated by using SAS 9.4 [(SAS Institute Japan Ltd.), EXSUS Version 8.1.0 (EPS Corporation)].

Experiment 1: Determination of the conditions of seeding PDX-derived tumor cells

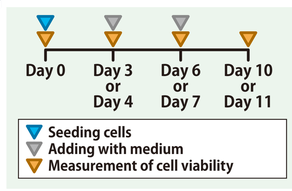
The primary tumor cells were dissociated from each PDX using cell disassociation materials. Some tumor cells were seeded into 3D culture plates and others were suspended with CellBanker 1 and frozen in liquid nitrogen.

The frozen tumor cells derived from each PDX were thawed and seeded into 3D culture plates on another day.

● Statics analysis

Cell division time = $Log_2 \{(T_D-B_D)/(T_0-B_0)\}$

T_D: The individual LI of control group on an arbitrary day
B_D: The mean LI of the blank group on an arbitrary day
T_0: The mean LI of the control group on Day 0
B_0: The mean LI of the blank group on Day 0
n = 3 wells
LI: Luminescence Intensity
Blank: medium without tumor cells
The day of seeding cells is defined as Day 0



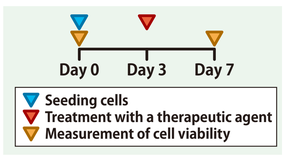
Experiment 2: Evaluation of the effects of therapeutic agents against cryopreserved PDX-derived tumor cells

CO-043-LSIM	5-FU (1.56 to 400 μM)	Irinotecan (0.391 to 100 μM)
GA-007-LSIM	Cisplatin (0.391 to 100 μM)	Trastuzumab (0.781 to 200 μg/mL)
LU-031-LSIM	Gefitnib (0.781 to 200 μM)	Cisplatin (0.391 to 100 μM)

● Statics analysis

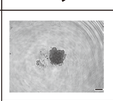
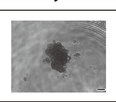
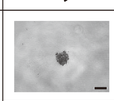
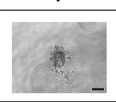
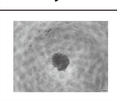
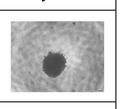
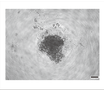
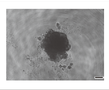
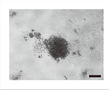
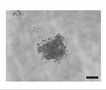
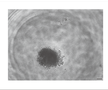
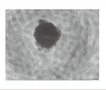
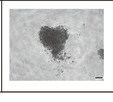
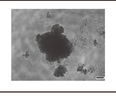
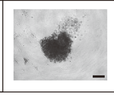
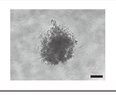
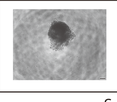
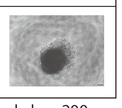
Cell Proliferation rate (%) = $\{(T_7-B_7)/(C_0-B_0)\}/\{(C_7-B_7)/C_0-B_0)\}\times100$

T: LI of each well on Day 7
C_7: The mean LI of the control group on Day 7
B_7: The mean LI of the blank group on Day 7
C_0: The mean LI of the control group on Day 0
B_0: The mean LI of the blank group on Day 0
n = 3 wells



Results

Fig. 1 Spheroids consisted of PDX-derived primary tumor cells

Seeded Cells (/well)	CO-043-LSIM 0.5% (v/v) Matrigel		GA-007-LSIM 0.5% (v/v) Matrigel		LU-031-LSIM 2% (v/v) Matrigel	
	Day 7	Day 11	Day 7	Day 11	Day 6	Day 10
1000						
2500						
5000						

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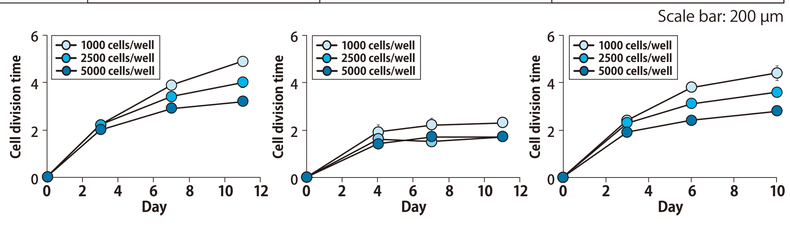
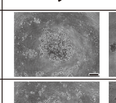
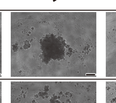
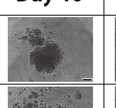
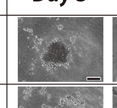
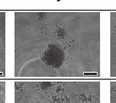
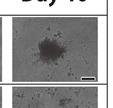
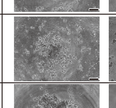
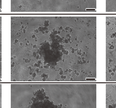
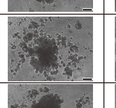
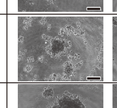
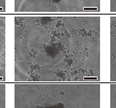
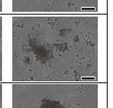
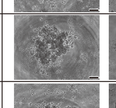
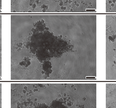
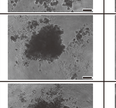
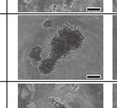
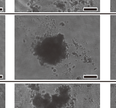
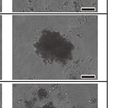
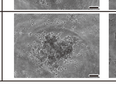


Fig. 2 The optimal conditions for 3D culture of cryopreserved PDX-derived tumor cells

Seeded Cells (/well)	Concentration of Matrigel (v/v)	CO-043-LSIM Day 3	CO-043-LSIM Day 7	CO-043-LSIM Day 10	GA-007-LSIM Day 3	GA-007-LSIM Day 7	GA-007-LSIM Day 10
2500	1%						
	2%						
5000	1%						
	2%						

Seeded Cells (/well)	Concentration of Matrigel (v/v)	LU-031-LSIM Day 4	LU-031-LSIM Day 7	LU-031-LSIM Day 11
1000	0.5%			
	2.0%			
2500	0.5%			
	2.0%			

Scale bar: 200 μm

Scale bar: 200 μm

Fig. 3 Effects of therapeutic agents against tumor cells derived from CO-043-LSIM

The number of seeded cells: 2500 cells/well, Matrigel concentration 1% (v/v)

5-FU	Assay No. 1	Assay No. 2	Assay No. 3
IC_{50} (μM)*	130	N.C.	N.C.
	82.6-204	-	-
E_{min} (%)	26.1	-	-
E_{max} (%)	75.9	-	-

Irinotecan	Assay No. 1	Assay No. 2	Assay No. 3
IC_{50} (μM)*	N.C.	N.C.	N.C.
	-	-	-
E_{min} (%)	-	-	-
E_{max} (%)	-	-	-

N. C.: Not calculated *: 95% confidential interval

The picture of Day 7

Scale bar: 200 μm

Fig. 4 Effects of therapeutic agents against tumor cells derived from GA-007-LSIM

The number of seeded cells: 2500 cells/well, Matrigel concentration 1% (v/v)

Cisplatin	Assay No. 1	Assay No. 2	Assay No. 3
IC_{50} (μM)*	12.0	10.7	14.3
	9.42-15.3	9.60-12.0	12.6-16.2
E_{min} (%)	-2.9	-2.1	-0.4
E_{max} (%)	99.2	104.4	103.6

*: 95% confidential interval

The IC_{50} of Trastuzumab was not calculated because the cell proliferation rate was not less than 50% on all the concentration groups.

The picture of Day 7

Scale bar: 200 μm

Fig. 5 Effects of therapeutic agents against tumor cells derived from LU-031-LSIM

The number of seeded cells: 1000 cells/well, Matrigel concentration 2% (v/v)

Gefitinib	Assay No. 1	Assay No. 2	Assay No. 3
IC_{50} (μM)*	11.2	13.6	12.9
	9.26-13.5	12.3-15.1	11.2-14.9
E_{min} (%)	-4.4	1.2	2.1
E_{max} (%)	134.3	105.7	108.8

Cisplatin	Assay No. 1	Assay No. 2
IC_{50} (μM)*	2.15	3.61
	1.57-2.95	2.39-5.44
E_{min} (%)	-2.0	-2.3
E_{max} (%)	94.9	99.1

*: 95% confidential interval

The picture of Day 7

Scale bar: 200 μm

Conclusion

● **The tumor cells derived from three kinds of PDXs could be cultured in 3D, *in vitro* [Fig. 1].**

We investigated whether the PDX-derived tumor cells can be cultured in vitro. The data of Fig. 1 shows that tumor cells derived from CO-043-LSIM, GA-007-LSIM, and LU-031-LSIM formed spheroids, and cell proliferation was observed.

● **The cryopreserved PDX-derived tumor cells could be cultured in 3D, and the optimal conditions for 3D culture of each PDX were determined [Fig. 2].**

We investigated whether not only primary but also cryopreserved PDX-derived tumor cells can be cultured in vitro. The data of Fig. 2 shows that cryopreserved PDX-derived tumor cells formed spheroids, and cell proliferation was observed. It was found that the number of seeded cells and the concentration of Matrigel were important factors for the PDX-derived tumor cells to form spheroids. Their optimal conditions varied for each PDX. Meanwhile, tumor cells derived from each PDX formed spheroids on Day 7 and Day 10.

● **The evaluation of anti-tumor effects of therapeutic agents against these PDXs was conducted [Fig. 3 to 5].**

We evaluated the anti-tumor effects of therapeutic agents against cryopreserved PDX-derived tumor cells. In the tumor cells derived from CO-043-LSIM, the cell viability following treatment of 5-FU or irinotecan decreased in a dose-dependent manner. In those derived from GA-007-LSIM, the cell viability following treatment of cisplatin decreased and its IC_{50} was from 10.7 to 14.3 μM. Meanwhile, the cell viability following treatment of trastuzumab did not decrease. In those derived from LU-031-LSIM, the cell viability following treatment of gefitinib or cisplatin decreased. IC_{50} of gefitinib was from 11.2 to 13.6 μM and that of cisplatin was 2.15 and 3.61 μM.

● **CONCLUSION**

We have determined how to evaluate the anti-tumor effects of therapeutic agents with the PDX-derived tumor cells. The evaluation system we have established may play a role in predicting the anti-tumor effects of therapeutic agents against PDX *in vivo*.