Establishment of *in vitro* 3D anti-tumor drug screening systems using PDCs spheroids

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Summary in Japanese

PDC（Patient–Derived Cell Line）は，がん患者の腹水より回収した細胞を低継代数で保存したものであり，抗がん剤の開発において臨床試験の薬剤予測率を高めるツールとして考えられている．また，がんの特徴を保持してその予測性を高める培養方法として，スフェロイド培養法（3次元）がある．3次元培養用の96穴プレートを用いて膵臓がんPDC，胃がんPDCそれぞれを播種したところ，胃がんPDCにおいてスフェロイド形成が認められた．さらにスフェロイド状の胃がんPDCにシスプラチンとドセタキセルをそれぞれ添加した．2次元培養に比べて3次元培養下の胃がんPDCのシスプラチンへの抵抗性は変わらなかったが，ドセタキセルへの抵抗性は上昇した．以上の結果から，3次元培養下の胃がんPDCを用いた試験系は，生体内のがんの薬剤抵抗性を考慮して，抗がん剤の薬効を評価できる可能性がある．

Objective

Patient-Derived Cell Lines (PDCs) have been established from the ascites of cancer patients and stocked after low-passage cultures (Tanaka Y, Chiwaki F. et al. Nat Cancer 2021). Since PDCs maintain the characteristics of the original cancers, they have been well-accepted as clinically reliable tools. *In vitro* screening systems using PDCs may be useful for anticancer drug screenings. In conventional *in vitro* studies, anticancer drugs are evaluated by two-dimensional (2D) cell culture; however, the characteristics of cells in 2D are different in some points from the original cancers. In this study, we aimed to develop drug screening systems with PDC-derived spheroids by three-dimensional (3D) cell culture which can partly mimic the original cancers. Gastric and pancreatic PDCs were seeded into microplates for 3D culture, and it was found that gastric PDCs formed spheroids. Furthermore, we evaluated anticancer drug efficacy with spheroids consisted of cells derived from gastric PDCs.

Materials and Methods

● PDCs

Pancreatic PDCs[a] : NPC-7C, NPC-20C
Gastric PDCs[a] : NSC-11C, NSC-14C, NSC-22C

a) National Cancer Center (NCC) supplied.

● Spheroid culture plate

Prime Surface 96U plate (Sumitomo Bakelite Co., Ltd.)

● Culture conditions

Cells were cultivated statically in a CO_2 incubator set at 37.0℃ and 5.0% CO_2 under a humidified condition.

● Anticancer drugs

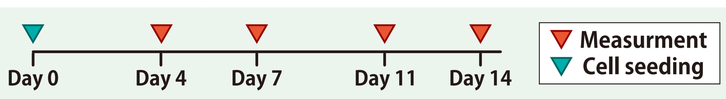
Cisplatin[b] : 0.391 to 100 μM (common ratio 2)
Docetaxel[b] : 3.91 to 1000 pM (common ratio 2)

b) Tokyo Chemical Industry Co., Ltd.

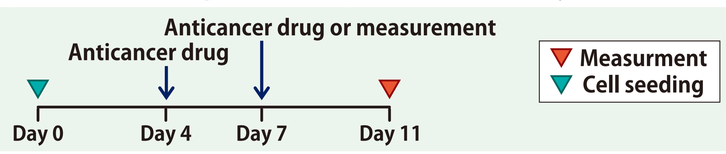
● Evaluation of cell viability

CellTiter-Glo 3D Cell Viability Assay (Promega Corporation)

● Experimental design (1) : Cell division times



● Experimental design (2) : Cell proliferation assay



● Calculation of cell division times, cell proliferation and IC_{50}

Cell division times = Log_2 {(D – B) / (D_4 – B_4)}

D: Luminescence intensity (LI) of Day 4, 7, 11 or 14
B: Mean LI value of the blank group
D_4: Mean LI value of Day 4
B_4: Mean LI value of the blank group of Day 4

Cell proliferation rate (%) = 100 × (T – B) / (C – B)

T: LI of anticancer drug
B: Mean LI value of the blank group
C: Mean LI value of the control group

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

Conclusion

● Optimization of 3D culture

The method of 3D culture of gastric PDCs is determined. The gastric PDCs proliferation increased gradually until 11 days after seeding (**Fig 3**). A seeding density of 500 cells/well resulted in increase of the number of dividing cells (**Fig 3**). These data show that the seeding 500 cells/well and the 3D culture duration of 11 days are appropriate conditions for investigating anticancer effect against gastric PDCs in this study. To find the method of 3D culture of pancreatic PDCs, further study is required.

● Anticancer drug against gastric PDCs in 3D culture

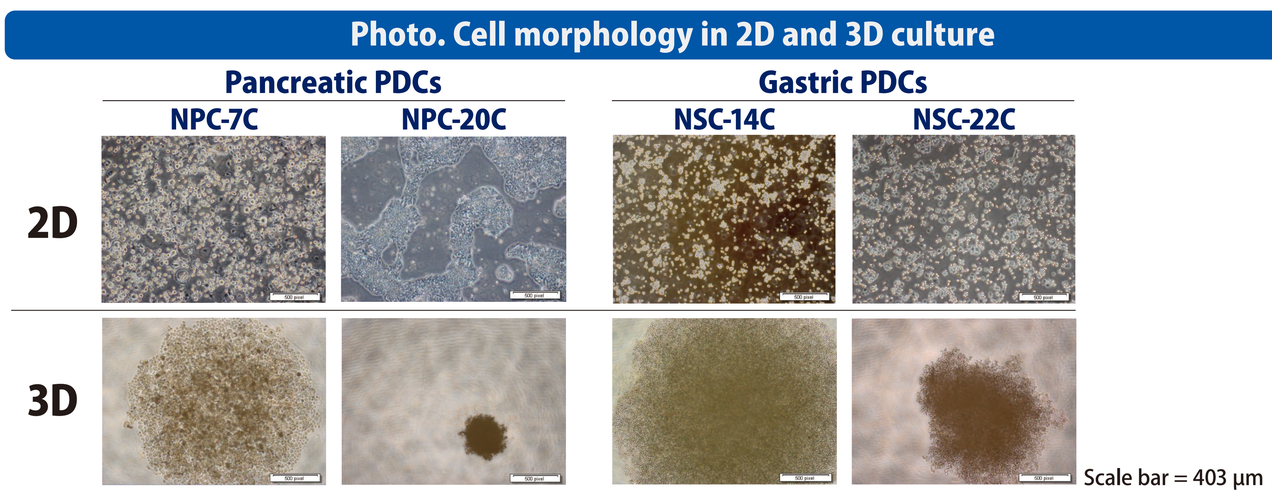
It has been reported that 3D culture models are more chemoresistant and clinically relevant than 2D (Seidlitz T et al. 2019; Nowacka M et al. 2021). Our results show that gastric PDCs had higher resistant to Docetaxel in 3D culture than in 2D culture (**Fig 5D**). On the other hand, IC_{50} of Cisplatin against gastric PDCs in 3D culture were similar to the one in 2D culture (**Fig 4D**). These data show that the gastric PDCs under 3D culture can maintain resistance to some kinds of anticancer drug. Our drug screening system with gastric PDCs in 3D culture can be better at predicting anticancer drug effect than a conventional drug screening system with gastric cancer cell lines in 2D culture.

* The data about 2D culture was reported at the 79th annual meeting of the Japanese cancer association.
(Title: Growth Inhibitory Effect of Anti-cancer drug against Human Peritoneal Metastatic Pancreatic Cancer Cell Lines)

Cell proliferation rate of 2D culture (%) = 100 × [(T – B4) – D1] / [(C4 – B4) – D1]
T: LI of anticancer drug, C4: Mean LI value of the control group of Day 4, B4: Mean LI value of the blank group of Day 4, B1: Mean LI value of the blank group of Day 1, C1: Mean LI value of the control group of Day 1, D1: LI of Day 1

Results

Photo. Cell morphology in 2D and 3D culture



Scale bar = 403 μm

Fig 1. Cell proliferation rate and IC_{50} in 3D culture for 7 days

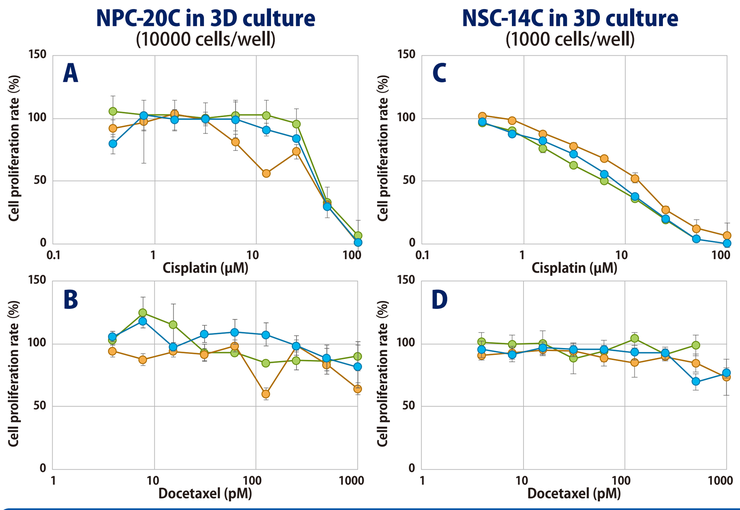


Table 1. IC_{50} of Cisplatin

IC_{50} (μM)	2D*	3D
NPC-7C	2.6	N.C
NPC-20C	72.5	41.6
NSC-14C	2.4	16.1

N.C: Not Calculated

Table 2. IC_{50} of Docetaxel

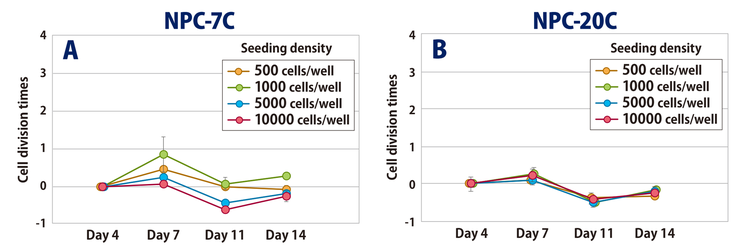
IC_{50} (nM)	2D*	3D
NPC-7C	0.194	N.C
NPC-20C	>1.00	N.C
NSC-14C	0.0245	N.C

N.C: Not Calculated

Data represent the mean ± standard deviations (n = 3) . The cell viability assay was conducted three times. (Each line shows each cell proliferation rate.)

Each IC_{50} in 3D culture was too high. We hypnotized that the duration of culture was too short to evaluate the effect of Cisplatin and Docetaxel.

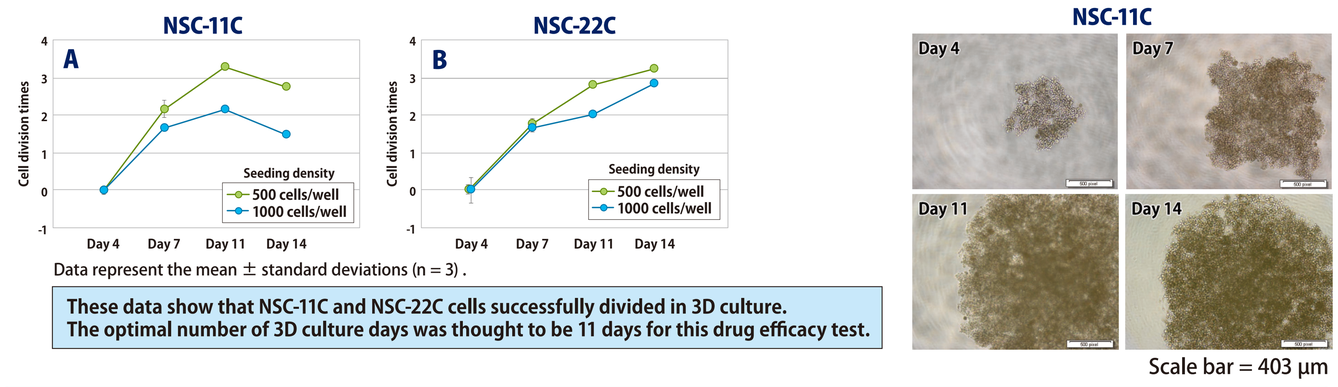
Fig 2. Cell division of pancreatic PDCs



Data represent the mean ± standard deviations (n = 3) .

These data show that NPC-7C and NPC-20C cells did not divide even in a 14-day 3D culture.

Fig 3. Cell division of gastric PDCs

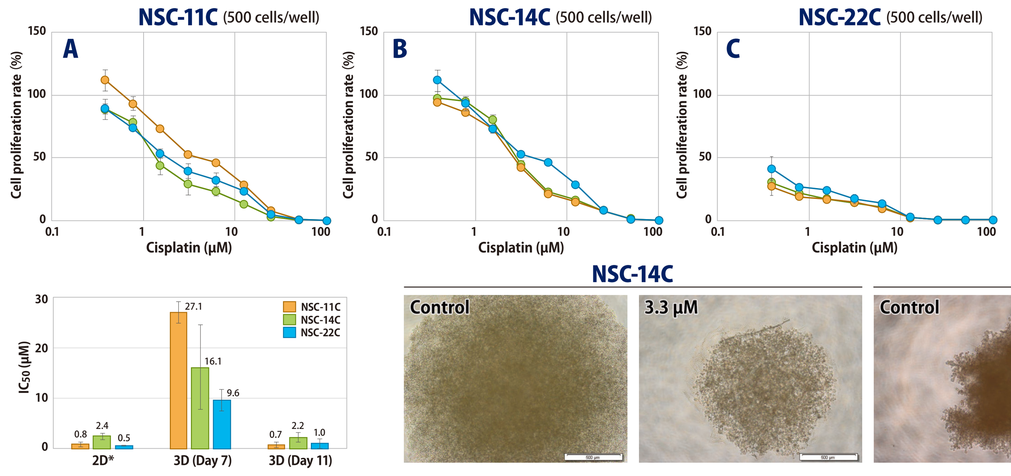


Data represent the mean ± standard deviations (n = 3) .

**These data show that NSC-11C and NSC-22C cells successfully divided in 3D culture.
The optimal number of 3D culture days was thought to be 11 days for this drug efficacy test.**

Scale bar = 403 μm

Fig 4. Cell proliferation rate and IC_{50} of Cisplatin against gastric PDCs in 3D culture for 11 days

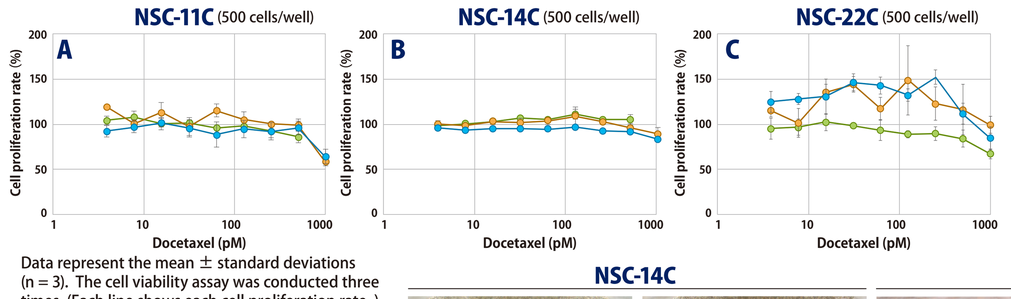


Extending the duration of culture from 7 to 11 days decreased IC_{50} of Cisplatin against gastric PDCs.

Data represent the mean ± standard deviations (n = 3) . The cell viability assay was conducted three times. (Each line shows each cell proliferation rate.)

Scale bar = 500 μm

Fig 5. Cell proliferation rate and IC_{50} against Docetaxel for gastric PDCs in 3D culture for 11 days



Data represent the mean ± standard deviations (n = 3). The cell viability assay was conducted three times. (Each line shows each cell proliferation rate.)

IC_{50} of Docetaxel against gastric PDCs in 3D culture was higher than the one in 2D culture.

Table 3. IC_{50} of Docetaxel

IC_{50} (nM)	2D*	3D
NSC-11C	0.0291	N.C
NSC-14C	0.0245	N.C
NSC-22C	0.0369	N.C

N.C: Not Calculated

Scale bar = 500 μm