Investigation of drug evaluation model using Patient-Derived Cell (PDC)

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Summary in Japanese

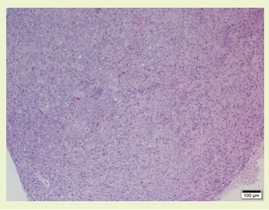
抗がん剤開発には，*in vitro*で長期継代されたがん細胞株を移植した担がんマウスが標準的な薬効評価モデルとして使用されている．しかし，これらのモデルで開発された新薬候補は，非臨床研究の結果と臨床研究の結果が一致しないものが多く存在することが課題となっている．そこで，我々はより臨床予測性の高い抗がん剤評価モデルの確立を試みた．

本実験で用いたPDC（Patient–Derived Cell）は，胃がんもしくは膵臓がん患者の腹水より回収した細胞で，国立がん研究センターから入手した．低継代数のPDCをマウスに移植して担がんモデルを作製し，既存の抗腫瘍薬を投与して腫瘍体積の変化により抗腫瘍効果を評価した．その結果，膵臓がんPDCモデルはゲムシタビン，胃がんPDCモデルはシスプラチンに対して，薬効に違いが認められた．

以上の結果から，PDCを用いた薬効評価モデルの確立は，既存の薬剤に抵抗性を示す癌の治療薬開発に有用であると考えられる．

Objective

HE staining of tumor (PANC-1 cells)



In anti-tumor drug discovery, established tumor cell lines are generally used for efficacy evaluation, but use of xenograft models inoculated with tumor cell lines cultured *in vitro* for long-term often results in a poor clinical prediction. A primary tumor consists of not only malignant cells but also non-malignant cells including stromal, immune and vascular cells, while established cell lines form a tumor mass composed of homogeneous cells (see the right figure). As a result, preclinical animal models show dissimilar properties to those of primary tumors, which is considered a cause of limited efficacy of drug candidates in clinical studies. To address this issue, we attempted to establish a drug evaluation system using patient-derived cancer cell (PDC)-inoculated models. PDCs from the ascites of patients with gastric or pancreatic cancer were obtained from the National Cancer Center (NCC), and were subcutaneously inoculated into immunodeficient mice in low-passage culture condition (≤20 passages). Two lines each of gastric and pancreatic PDCs were used in the efficacy evaluation of pre-exiting anti-tumor drugs. In addition, we investigated the expression of cancer stem cell markers, CD44 and CXCR4 by immunohistochemistry (IHC).

Materials and Methods

● PDCs

PDCs collected from the ascites of gastric cancer patients (NSC-10C, NSC-14C and NSC-39C) and pancreatic cancer patients (NPC-7C and NPC-20C) were obtained from the NCC. PDCs were incubated *in vitro* and prepared for inoculation by suspending in DPBS and matrigel.

● Reagents

Cisplatin (CDDP), (Product name: Randa Injection, Nippon Kayaku Co., Ltd.)
Capecitabine (Cape), (Product name: Xeloda, Chugai Pharmaceutical Co., Ltd.)
Docetaxel (DTX), (Product name: ONETAXOTERE I.V. Infusion, Sanofi K.K.)
Gemcitabine (Gem), (Product name: Gemzar Injection, Eli Lilly Japan K.K.)

● Animal experiment

Female nude mice and SCID Beige mice were used for the experiment of gastric cancer and pancreatic cancer, respectively. All animals were purchased from The Jackson Laboratory Japan, Inc. PDCs were subcutaneously inoculated in the right abdominal region of each animal.
Tumor diameter was measured using caliper and the volume was calculated by the following equation.

Estimated tumor volume (mm^3) =
1/2 × long diameter (mm) × short diameter (mm) × short diameter (mm)

Tumor-bearing animals were assigned homogeneously to each test group by the "stratified randomization method" on the basis of the tumor volume.
After group allocation, tumor diameter and body weight were measured twice a week.
Tumor growth inhibition [TGI (%)] was calculated as follows:

TGI (%) = (1 – DV / CV) × 100

DV: the mean of tumor volume in the drug treatment groups,
CV: the mean of tumor volume in the control group

● HE staining and IHC

Paraffin-embedded blocks were created from tumor immersed in 10% formalin neutral buffer solution. Then, HE staining and IHC were performed. Anti-CD44 (ab157107, abcam) and anti-CXCR4 (ab124824, abcam) antibodies were used.

Discussion

In long-term 2D culture, tumor cell lines often lose their heterogeneity, accumulate further gene mutations and adapt to the artificial culture condition. As a result, tumor cell line-inoculated xenografts show dissimilar biological properties to those of primary tumors, which is considered a major hurdle for development of antitumor drugs. As a high predictive model, patient-derived xenograft (PDX) is currently used for drug discovery. PDX, which is produced by inoculation of a surgically excised tumor from a patient to mice (or passage from PDX mice to other mice), contains non-malignant cells and maintains the tumor microenvironment. Therefore, PDX is highly likely to show a similar response against drugs as primary cancers and is thus deemed to be a beneficial tool for drug discovery. On the other hand, unresectable metastatic cancer is often associated with worse prognosis and is an essential target of chemotherapy. We assumed that PDCs from metastatic caners such as ascites carcinoma provide a new option for drug discovery. Indeed, PDCs from the ascites of patients with gastric and pancreatic cancers in this study showed differential responses against cisplatin and gemcitabine, respectively. Therefore, it is suggested that PDC-inoculated models are useful as good predictive models for antitumor drug discovery.

Results

Gastric PDCs

NSC-10C

As an example of the efficacy and the resistance against a single drug

Photos *in vitro*

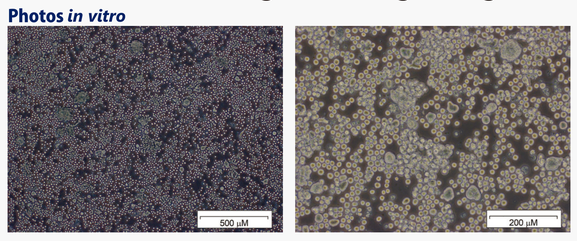


Fig 1. Tumor growth in NSC-10C-bearing nude mice

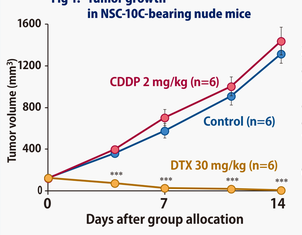
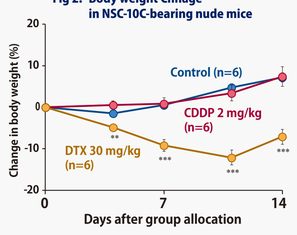
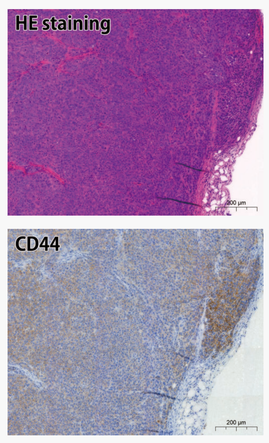


Fig 2. Body weight Chnage in NSC-10C-bearing nude mice



Points: mean for 6 animals; vertical bars: standard error
**: p<0.01, ***: p<0.001 vs the Control group (Student's t test).

CDDP or saline was administered on Days 0 and 7 (i.v.).
DTX or saline was administered on Days 0 and 4 (i.v.).



TGI (%) in the NSC-10C-inoculated model

Group	No. of animal	TGI (%) (Day 14)
Control	6	-
CDDP 2 mg/kg	6	-10.3
DTX 30 mg/kg	6	97.9

NSC-14C

As an example of a cachexia-like model and the additive effect of drug combination

Photos *in vitro*

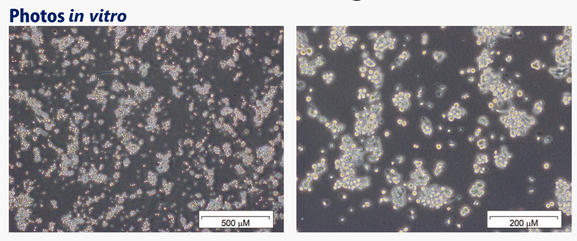


Fig 3. Tumor growth in NSC-14C-bearing nude mice

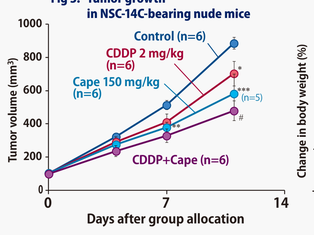
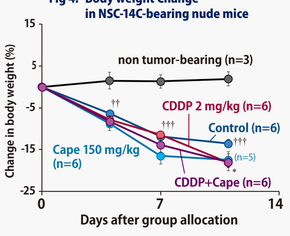
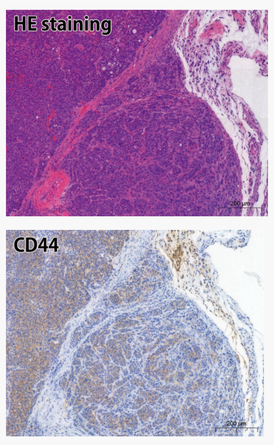


Fig 4. Body weight Change in NSC-14C-bearing nude mice



Points: mean for indicated animals; vertical bars: standard error
†† : p<0.01, ††† : p<0.001 vs the non tumor-bearing group (Student's t test).
*: p<0.05, **: p<0.01, ***: p<0.001 vs the Control group (Student's t test).
#: p<0.05 vs the CDDP 2 mg/kg group (Student's t test).

CDDP or saline was administered on Days 0 and 7 (i.v.).
Cape or 0.5w/v% methyl cellulose was administered once a day from Day 0 to Day 11 (p.o).
An animal in the Cape 150 mg/kg group was reached humane endpoint because of emaciation and euthanized on Day 7.



TGI (%) in the NSC-14C-inoculated model

Group	No. of animal	TGI (%) (Day 11)
Control	6	-
CDDP 2 mg/kg	6	20.7
Cape 150 mg/kg	5	34.4
CDDP + Cape	6	46.0

Pancreatic PDCs

NPC-7C

As an example of the tumor regression by a single drug

Photos *in vitro*

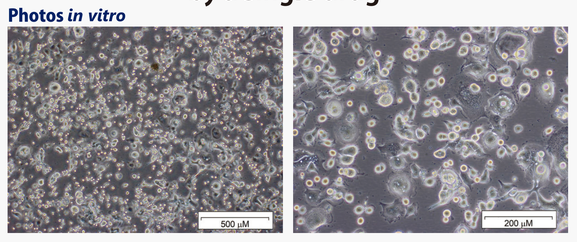


Fig 5. Tumor growth in NPC-7C-bearing SCID Beige mice

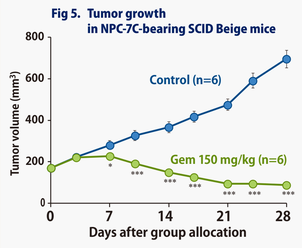
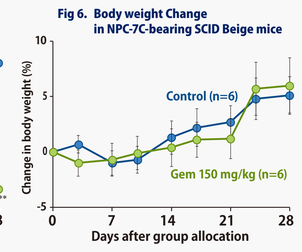
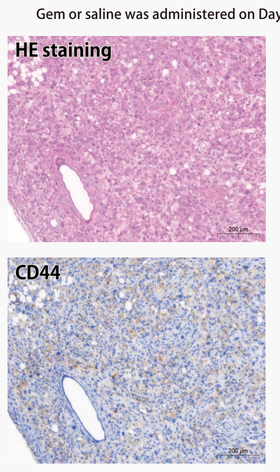


Fig 6. Body weight Change in NPC-7C-bearing SCID Beige mice



Points: mean for 6 animals; vertical bars: standard error
*: p<0.05, ***: p<0.001 vs the Control group (Student's t test).

Gem or saline was administered on Days 0 , 3, 7, 10, 14 and 17 (i.v.).



TGI (%) in the NPC-7C-inoculated model

Group	No. of animal	TGI (%) (Day 28)
Control	6	-
Gem 150 mg/kg	6	87.5

NPC-20C

As an example of the tumor growth inhibition by a single drug

Photos *in vitro*

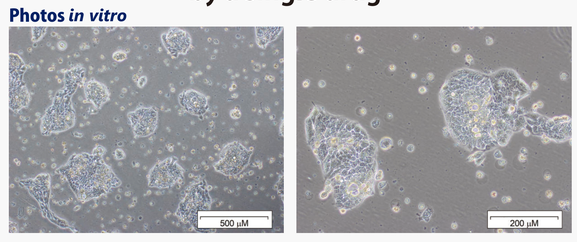


Fig 7. Tumor growth in NPC-20C-bearing SCID Beige mice

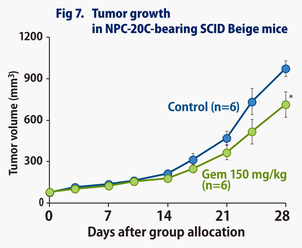
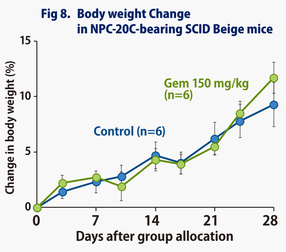


Fig 8. Body weight Change in NPC-20C-bearing SCID Beige mice



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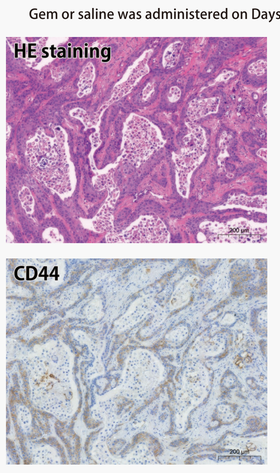
Gem or saline was administered on Days 0 , 3, 7, 10, 14 and 17 (i.v.).



TGI (%) in the NPC-20C-inoculated model

Group	No. of animal	TGI (%) (Day 28)
Control	6	-
Gem 150 mg/kg	6	26.7

NSC-39C As an example that were not observed tumor engraftment