

Experimental Pancreatic Cancer Model Using PGHAM-1 Cells: Characteristics and Experimental Therapeutic Trials

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Abstract

We developed short-term pancreatic cancer models in hamsters using PGHAM-1 cells and examined the utility of the models for research on metastasis and for therapeutic trials. With 3 PGHAM-1 models, including 1) primary pancreatic cancer and simultaneous liver metastasis by intrapancreatic transplantation, 2) liver metastasis alone by intrasplenic transplantation, 3) peritoneal dissemination by intraperitoneal transplantation, within 21 days after inoculation, we studied the specific characteristics of metastases and the effects of several antiangiogenic substances on primary and metastatic pancreatic tumors. Several experiments showed that vascular endothelial growth factor and anatomical characteristics were important factors for metastasis. In therapeutic experiments, the incidence, size, diameter, microvessel density, and apoptotic index of the tumors were preferably influenced by the antiangiogenic substances. In addition, PGHAM-1-Luc, which is luciferase-positive PGHAM-1 cell line, was newly developed and is expected to be a useful new animal model. These models would be suitable for the study of pathogenesis of pancreatic cancer and its metastasis and for preclinical trials of chemotherapeutic agents, such as antiangiogenic substances.

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Key words: pancreatic cancer, experimental model, PGHAM-1, therapeutic trial

Introduction

Because pancreatic cancer is difficult to detect at an early stage, it readily metastasizes to lymph nodes and the liver, spreads through the peritoneum, and recurs locally. Thus, the prognosis for pancreatic cancer is significantly worse than for other malignant conditions of the digestive organs. The current treatment focuses on surgery, but because the results have not been satisfactory there

is a demand for new methods of diagnosis and treatment¹. A range of fundamental research is being undertaken for pancreatic cancer, but new work on molecular biological techniques is only at the first stage of hypothesis verification, so it will be a long time before this research can be used with human models. Strategies will also be needed to apply the results to the clinical setting. To this end, experiments using animal models of disease play an essential role in making the leap from *in vitro* to *in vivo* or from *in vivo* to clinical application, and a

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range of experimental models have been devised for pancreatic cancer². This paper gives an overview of the experimental models for pancreatic cancer and also discusses the importance of our experimental model, which uses PGHAM-1 cells to control metastasis.

Animal Models for Pancreatic Cancer

There are several animal models of pancreatic cancer, but the most well-known is the hamster model of pancreatic cancer described by Pour *et al*³. In the study of Pour *et al*, the carcinogenic nitroso compound N-nitrosobis(2-oxopropyl)amine (BOP) was administered by subcutaneous injection into Syrian golden hamsters to stimulate the development of pancreatic cancer. The resultant cancer was a well-differentiated adenocarcinoma that was extremely morphologically similar to its human counterpart and had the same biological characteristics, such as expression of *K-ras* and other oncogenes; for these reasons it has been used to study histogenesis in pancreatic cancer. In this model, a single dose or multiple doses of BOP can induce pancreatic cancer at a high rate, but approximately 20 weeks is needed for cancer to develop. Various mechanisms have been devised to reduce this interval⁴. Moreover, while metastases to the lymph nodes and the liver have been identified in this model, they do not occur frequently, and it takes a long time for the secondary cancer to develop; therefore, this model is not considered suitable for studies of metastasis.

In other animal models of pancreatic cancer, the chemical carcinogens azaserine and 4-hydroxyaminoquinoline-1-oxide (4-HAQO) are administered to rats to stimulate acinar cell carcinoma, and 7,12-dimethylbenz(a)anthracene (DMBA) is implanted directly into the pancreas to cause adenocarcinoma². There are many similar, but none can outdo the hamster model. We believe that the BOP pancreatic hamster model is currently the leader in this field.

The Characteristics of PGHAM-1 Cells:

At Nippon Medical School we were searching for

a model of pancreatic cancer metastasis that would cause a high rate of metastasis in a short time-frame. From a hamster in which BOP-induced pancreatic cancer tissue had been subcutaneously implanted, we established a transplantable pancreatic cancer cell line (PGHAM-1) and then performed a homologous transplantation of these cells to produce an experimental model that could cause both pancreatic cancer itself and metastases over a short period⁵. With a cell doubling time of 14.4 hours, PGHAM-1 has high proliferative potential. Furthermore, we have found that tumors formed by PGHAM-1 cells bear a close histological resemblance to human pancreatic cancer by having high invasive capability and high metastatic potential and also bear a close biological resemblance in having point mutations at *K-ras* codon 12, vascular endothelial growth factor (VEGF)⁶, and matrix metalloproteinase 2.9⁷. We have also demonstrated that homologous transplantation within the pancreas causes a high rate of liver metastasis from an early stage.

Experimental Models Using PGHAM-1 Cells:

There are 3 methods of developing a transplant model that uses PGHAM-1 cells: the pancreatic transplantation model, the splenic transplantation model, and the peritoneal transplantation model.

1) Pancreatic transplantation model: In this model 5×10^6 PGHAM-1 cells are transplanted into the splenic lobes of the pancreas of the same species of hamster to stimulate development of a primary pancreatic tumor and liver metastasis (**Fig. 1a and 2**).

2) Splenic transplantation model: In this model 1×10^6 PGHAM-1 cells are transplanted into the spleen of the same species of hamster to stimulate liver metastasis (**Fig. 1b and 3**).

3) Peritoneal transplantation model: In this model 1×10^6 PGHAM-1 cells are transplanted into the abdominal cavity to stimulate peritoneal dissemination (**Fig. 1c and 4**).

All 3 methods enable the formation of cancer within 21 days after transplantation (**Table 1**).

Leaving aside 1) the difference between the 2 species, human and hamster, and 2) the fact that the

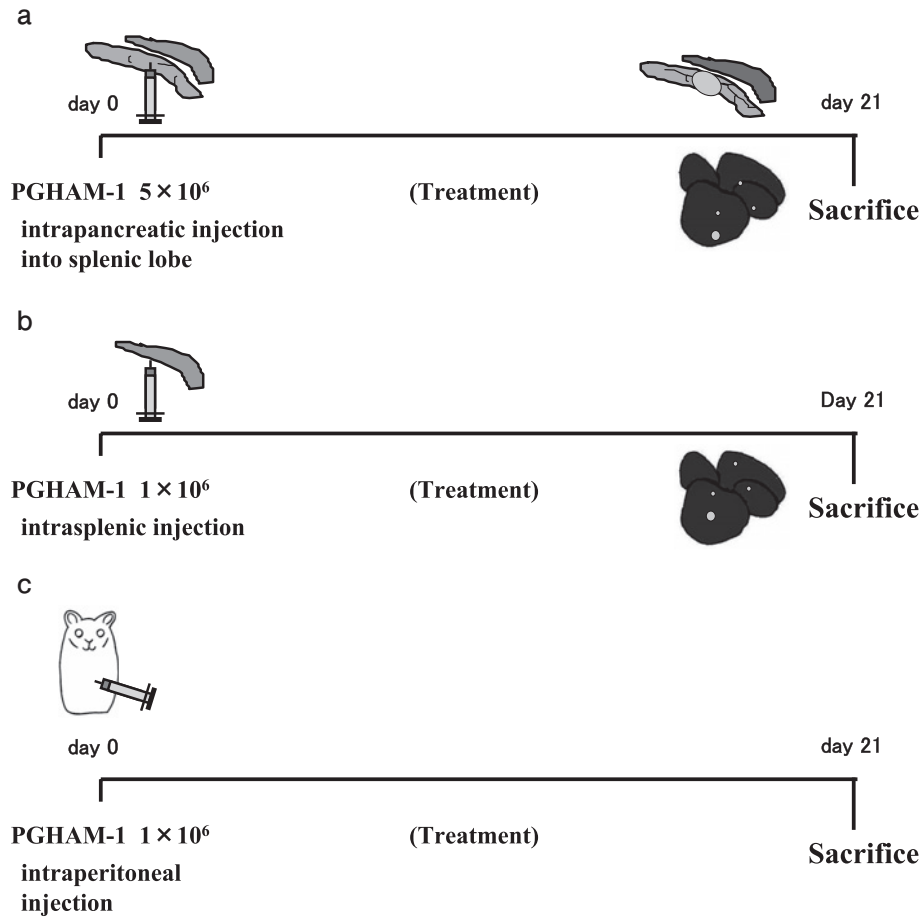


Fig. 1 Diagrams of 3 transplantation models using PGHAM-1 cells. **a:** The intrapancreatic transplantation model with PGHAM-1 cells induced pancreatic tumors in 100% of animals and liver metastasis in 40% to 80%. **b:** The intrasplenic transplantation model with PGHAM-1 cells induced liver metastasis in 100% of animals. **c:** The intraperitoneal transplantation model with PGHAM-1 cells induced peritoneal dissemination in 100% of animals.

metastasis does not originate from a pure carcinogenesis and considering that the immune system is also retained in the relationship between the host and the cancer cells, then in terms of tissue invasion and the morphology of metastasis, this model is more natural than previous experiments in metastasis in which human pancreatic cancer cells were transplanted into nude mice. Thus, we consider this model to be the best model of metastasis of pancreatic cancer.

Application of Understanding of the Characteristics of Metastasis:

Through observation of this model over time, we were able to develop an image of early metastasis. Focusing on angiogenesis, we examined the

relationship between expression of VEGF and liver metastasis. VEGF, for which staining in the primary pancreatic tumor was positive, was expressed at lower levels in the metastases in the liver. When the metastasized tumor was transplanted back into the pancreas, VEGF expression again increased. A likely reason for this increase is that because the liver has an abundant blood supply, cancer cells in the liver do not need to express VEGF, but for the cells to grow in the pancreas again, VEGF becomes necessary once more. This indicates that the condition of the liver as a metastatic site has a significant effect on the metastasis itself⁶.

Moreover, when the peritoneal dissemination model was used, an image of early peritoneal dissemination was easily obtained. In experiments to induce peritoneal metastasis of pancreatic cancer in

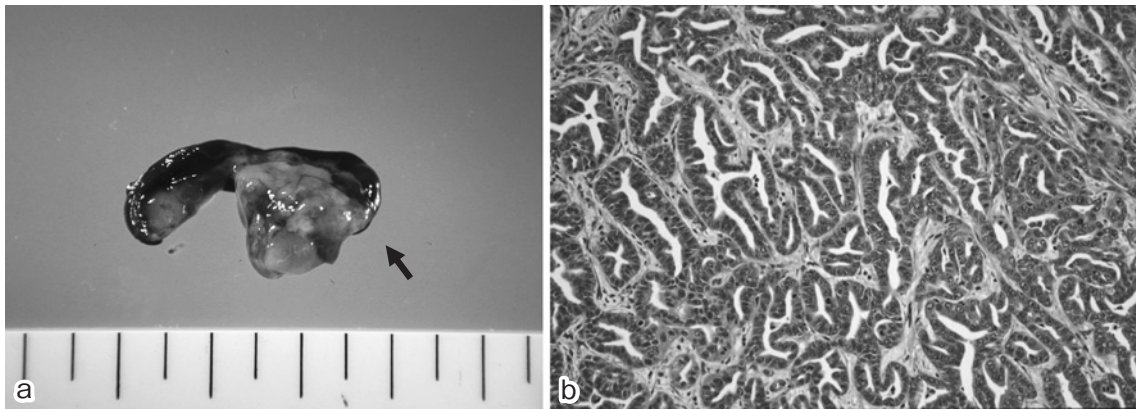


Fig. 2 Photographs of pancreatic tumor in the intrapancreatic transplantation model. **a:** A white nodule (↑) 5 mm in diameter was found in the splenic lobe of the pancreas. **b:** Histological examination showed well-differentiated adenocarcinoma.

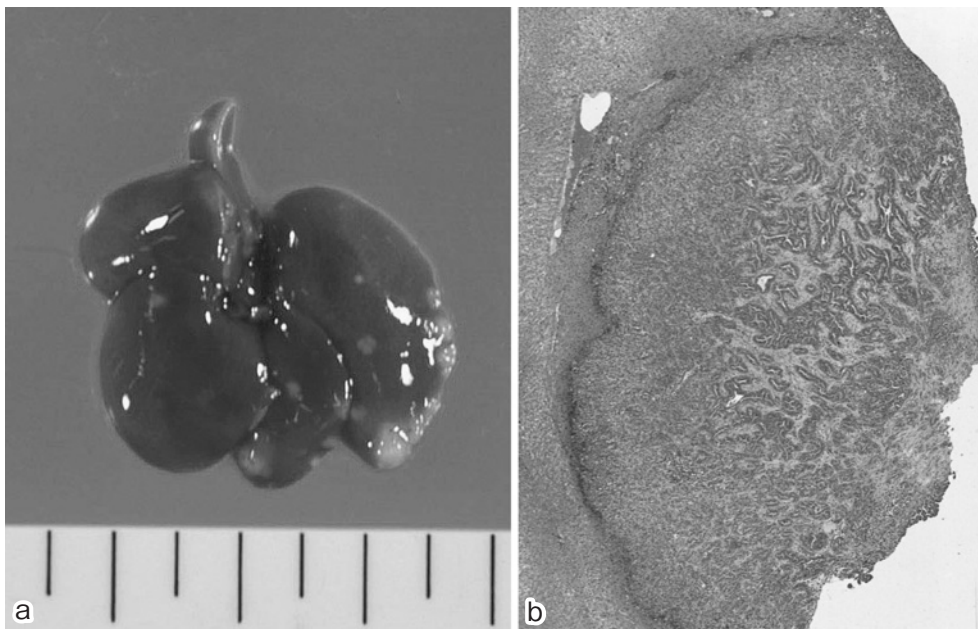


Fig. 3 Photographs of liver tumors in the intrasplenic transplantation model. **a:** Several white nodules were found. **b:** The histological type was well-differentiated adenocarcinoma, as was that of the primary tumor.

hamsters using PGHAM-1 cells, we discovered 2 different mechanisms: lymphatic metastasis in the diaphragm and greater omentum and direct adhesion in the parietal peritoneum. We were also able to demonstrate that each type of metastasis has its own particular process. In addition, by interposing anatomical absorption apparatuses, such as milky spots and stomata, in the beginning stage of metastasis, we were able to verify that the mechanism of lymphatic metastasis gave rise to metastases at an earlier stage than did the

mechanism of direct adhesion⁸.

For these reasons, we believe that this model is very useful as a means of advancing understanding of the characteristics of metastasis.

Application to Experimental Trials

As well as aiding understanding of the characteristics of metastasis, this model can also be used to examine the therapeutic benefits of various drugs because its biological characteristics are

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Table 1 Differences in tumors by induced 3 types of transplantation of PGHAM-1 cells

	Pancreatic tumor	Liver metastasis	Peritoneal dissemination
Intrapancreatic transplantation	++	+	±
Intrasplenic transplantation	-	++	-
Intraperitoneal transplantation	-	-	++

++ , incidence of 80% to 100%; + , incidence of 40% to 80%; - , not induced.

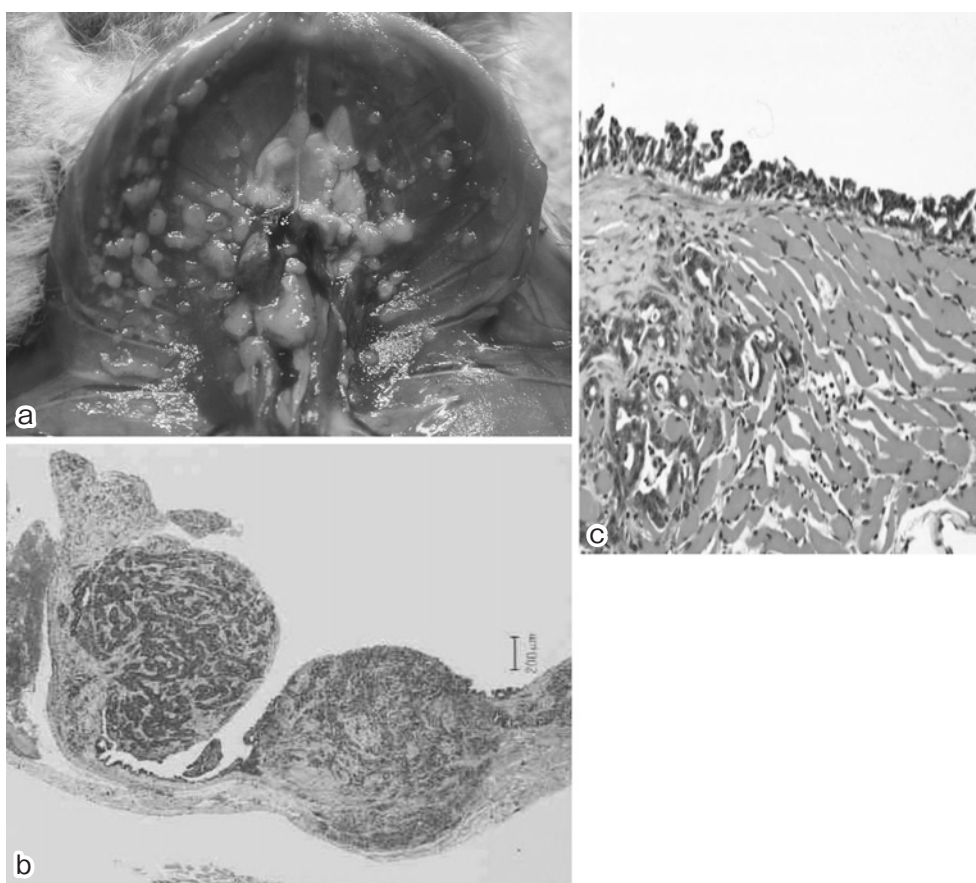


Fig. 4 Photographs of peritoneal disseminated tumors in the intraperitoneal transplantation model. **a:** Multiple macroscopic white nodules were found on the diaphragm. **b:** A lower-magnification photomicrograph of disseminated tumors on the diaphragm. **c:** Carcinoma cells were found in the diaphragm and the muscle layer.

similar to those of human pancreatic cancer and pancreatic tumors and metastases are quickly produced. An example of an application of the model is the examination of the effects of the angiogenesis inhibitor angiostatin on pancreatic cancer, as follows. The transplant model selected was the 1 of the 3 described above considered to be most appropriate for the drug in question; for angiostatin the selected

model was the liver metastasis model. As shown in **Figure 5**, we administered angiostatin, and 21 days after transplantation we investigated the following variables: the rate of metastasis, the greatest dimension of the metastasized tumor, microvessel density (MVD) (staining for Factor VIII-related antigen) and the apoptotic index (TUNEL assay). The results demonstrated that angiostatin

Table 2 Effects of various antiangiogenic substances in pancreatic transplantation models with PGHAM-1 cells

Agents	Models	Pancreas	Liver	Peritoneum	MVD	AI
Angiostatin ⁹⁾	i.s	–	↓	–	↓	↑
TNP-470	i.panc.	↓	↓	–	NE	NE
Thalidomide	i.p.	–	–	↓	↓	↑
Tranilast ¹⁰⁾	i.p.	–	–	↓	↓	NE
MMI-166 ⁷⁾	i.panc.	↓	↓	–	↓	↑

i.s: intrasplenic, i.panc.: intrapancreatic, i.p.: intraperitoneal, AI: apoptotic index, NE: not examined, ↓: decreased incidence of tumors. ↑: increased incidence of tumors, –: not induced.

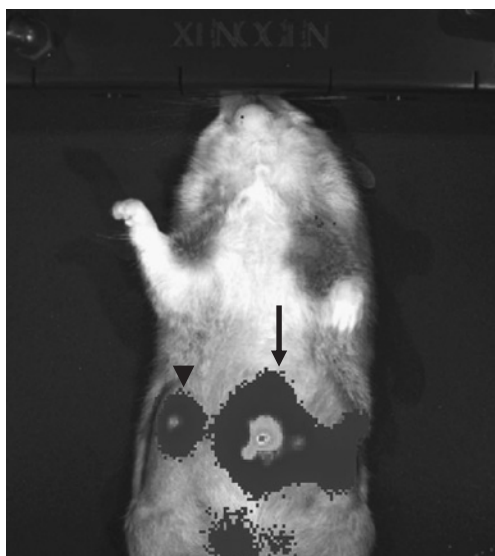


Fig. 6 In vivo observation of pancreatic tumor (↓) and its liver metastasis (▼) by intrapancreatic transplantation of PGHAM-1-Luc tumor (5×10^6) with the IVIS imaging system (IVIS 100 System, Xenogen, Alameda, CA USA).

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