

## Comparison of Metastatic Behavior of Parental and Metastatic Pancreatic Cancer Cell Lines in Syrian Golden Hamsters

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**Abstract.** *The major cause of death in patients with pancreatic cancer is metastatic disease. In fact, at the time of diagnosis, patients usually have locally advanced or metastatic disease involving lymph nodes, liver, lungs or peritoneum. Therefore, for a better understanding of the tumoral behavior to design prevention or treatment strategies, in vivo models are important. We report here the results of the metastatic behavior of parental and metastatic cell lines in a hamster pancreatic cancer model when implanted orthotopically (OIH,OIM) or into the liver (LIH,LIM). Metastatic sites and survival were studied. Survival ranged from 72 to 105 days. The OIH group showed spontaneous metastases to lymph nodes. The second target organ was the lung. Liver metastases appeared earlier in the OIM group than OIH. LIH showed only metastases to the pancreas while the LIM group showed metastases to pancreas, vas deferens and testis. This study suggests that the metastatic behavior of parental and metastatic cell lines is different. Thus, this should be considered in the planning of clinical or surgical treatment against pancreatic cancer.*

Pancreatic adenocarcinoma is a highly aggressive malignancy that shows great resistance even to a surgical approach. This aggressive behavior may be due to a highly patterned set of biologic features for its development (1-3).

The major cause of death in patients with pancreatic cancer is metastatic disease. In fact, at the time of diagnosis, patients usually have locally advanced or metastatic disease involving lymph nodes, liver, lungs or even peritoneum (4-6).

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**Key Words:** Metastatic experimental model, orthotopic implantation, intrahepatic implantation, pancreatic cancer, Syrian golden hamsters.

Therefore, for a better understanding of the tumoral behavior in order to design prevention or treatment strategies, *in vivo* models are important.

Hamster pancreas bears similarity to human pancreas phylogenetically, topographically, anatomically, genetically and immunologically (7-13). We have previously reported an orthotopic tissue implantation model where the liver was the metastatic target organ, resembling the clinical characteristics of the disease (14,15). We also have published about the "return trip" metastases from the liver to the pancreas, where the "seed and soil" was matched (16,17). However, the process of metastasis of pancreatic cancer *in vivo* using Syrian golden hamsters has never been reported. Thus, our purpose was to elucidate the metastatic behavior of parental and metastatic cell lines in a hamster pancreatic cancer model.

### Materials and Methods

**Experimental protocol.** Animals were divided randomly into 4 groups according to the organ of tumor graft implantation: i. orthotopic pancreatic implantation of HaP-T1 (OIH,n=10); ii. liver implantation of HaP-T1 (LIH,n=10); iii. orthotopic pancreatic implantation of MS-PaS-1 (OIM,n=10); and iv. liver implantation of MS-PaS-1 (LIM,n=10). Follow-up was done. Two animals from each group were sacrificed on Day 42, Day 49, Day 56 and Day 63 in the OIH and LIH groups and on Day 46, Day 49, Day 60 and Day 66 in the OIM and LIM groups. Survival time was studied on the remaining 2 hamsters of each group.

**Cell lines.** HaP-T1 and MS-PaS-1 were used in these experiments. HaP-T1 is a BHP-induced pancreatic cancer cell line, established by Saito *et al.* (18) and MS-PaS-1 is a pancreatic cancer cell line established from "return trip" metastases from liver to the pancreas (17). K-ras point mutation at codon 12 from GGT to GAT is present in both cell lines (14, 17).

**Animals.** Forty-four male Syrian golden hamsters (GN strain), aged 16.4 weeks (range:18-22), weighing 138 g (range: 136-156) were used. They were maintained in the Laboratory Animal Center of Toyama Medical and Pharmaceutical University, Japan, under 12 h/12 h light/dark cycle, fed commercial chow and water *ad libitum*.

Table I. Outcome of the animals.

	OIH (n=10)	LIH (n=10)	OIM (n=10)	LIM (n=10)
Success of implantation	100%	100%	100%	100%
Survival time (days)	72	102	70	75
Metastatic sites				
Day 42	Ln,Ki	Pa	-	-
Day 46	-	-	VD,Te	VD,Te
Day 49	Lu,Ln	Pa	Li,VD,Te	Pa,VD,Te
Day 56	Pa,Lu,Ln	Pa	-	-
Day 60	-	-	Li,VD,Te	Pa,VD,Te
Day 63	Li,Ln,Te	Pa	-	-
Day 66	-	-	Li,others	Pa,others

OIH, Orthotopic pancreatic implantation of HaP-T1 (parental cell line); LIH, Liver implantation of HaP-T1; OIM, Orthotopic pancreatic implantation of MS-PaS-1; LIM, Liver implantation of MS-PaS-1. N=2 in each group of sacrifice. Ln, Lymph node; Ki, Kidney; Pa, Pancreas; VD, Vas Deferens; Te, Testis; Lu, Lung; others, include ascites, kidneys, lymph nodes and epididimis.

**Anesthesia.** Hamsters were anesthetized with diethyl-ether inhalation and pentobarbital (5 mg/kg / body-weight) intraperitoneally.

**Tumor cell suspensions for inoculation.** Subconfluent cultures were washed with phosphate-buffered saline (PBS) and harvested with trypsin 0.025% and EDTA 0.02%. The cell density was adjusted to 2 x 10<sup>6</sup> cells/ml. They were kept in cold serum-free medium until use.

**Preparation of tumor graft.** The cell suspension was inoculated subcutaneously in 4 hamsters (n=2, parental cell line and n=2, metastatic cell line). In the exponential phase of growth, the tumors were resected. Necrotic areas were removed and cut into 1 mm<sup>3</sup> fragments. They were kept in cold serum-free medium. The tumor pieces of each animal were implanted in 10 of each group, i.e., 5 orthotopically and 5 into the liver.

**Implantation of tumor grafts.** Orthotopic pancreatic implantation (OIH,n=10,OIM,n=10): After anesthesia, one piece of tumor was implanted into the splenic lobe of the pancreas and the hole was closed with Vycril® suture 7-0 (Ethicon Co., NJ, USA). The abdominal wall was closed in two layers with nylon 4-0 sutures (14,15). Liver implantation (LIH, n=10, LIM, n=10): After anesthesia, one fragment of graft was implanted into the frontal lobe of the liver and the hole was closed as described above (16,17).

**Follow-up and sacrifice of the animals for study.** Abdominal palpation was done and body-weight of the animals was recorded weekly. They were sacrificed with diethyl-ether inhalation. Necropsy was performed and specimens such as liver, pancreas, lungs, kidneys, lymph nodes, vas deferens, epididimis and testis were fixed in formalin for histopathological study. Specimens were also frozen in liquid nitrogen for DNA extraction in order to detect K-ras point mutation.

**Histopathological examinations.** Specimens were stained with hematoxylin eosin and alcian blue/PAS.

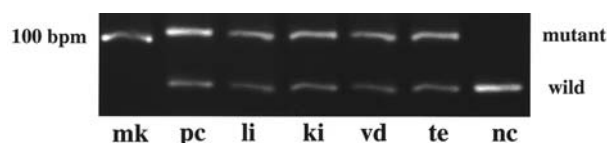


Figure 1. PCR/RFLP analysis of a case of the PIM group. Cancer shows two bands, a mutant and a wild. Normal tissue shows only a wild band. mk, marker; pc, positive control (DNA from HaP-T1); li, liver; ki, kidney; vd, vas deferens; te, testis; nc, negative control (DNA from a 12-week-old hamster liver).

**Detection of K-ras point mutation at codon 12 by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).** DNA was extracted with sodium dodecyl sulfate and proteinase K. The crude extract was purified using the phenol-chloroform-isoamylalcohol precipitation method. Primers used were as follows: sense 5'-ACT GAA TAT AAA CTT GTG GTA GTT GGA CCT-3' (19) and antisense 3'-TTA TAC TAG GAT GCT ATC TC-5' (20). PCR conditions were 92°C for 20 sec for denaturing, 50°C for 2 min for annealing, 72°C for 20 sec for extension, in a total of 35 cycles in 50 ml of PCR mixtures. For RFLP analysis, BstN-1 enzyme was used for 3 h at 60°C (14-16).

## Results and Discussion

During the follow-up and confirmation after necropsy, all groups showed tumor growth at the site of implantation. Therefore, the success rate of implantations was 100%. Survival time was in mean 72, 102, 70 and 75 days, in OIH, LIH, OIM and LIM, respectively (Table I). All necropsied specimens showed the presence of K-ras point mutation (Figure 1).

After sacrifice, tumors of the OIH group showed spontaneous metastases to lymph nodes on Day 42. These findings persisted in the following sacrifice days. The second target organ was the lung (Day 49). Thus, this phenomena appeared to be an early event in contrast to metastatic implanted groups. Liver metastases appeared earlier in the OIM group (Day 49) than OIH (Day 63) (Table I).

Comparing liver implanted groups, LIH showed only metastases to the pancreas from Day 42, coinciding with our previous report (16). On the other hand, the LIM group showed metastases to several organs. Thus, tumor cells from metastatic implanted groups showed similar tumoral behavior with similar preferences for metastatic sites and presented "return trip" metastases phenomena. Pancreatic implantation of metastatic cells has the liver as the "return trip" metastases organ while liver implanted cells showed metastases to the pancreas.

Considering metastatic cell line derived tumor implanted groups (OIM, LIM), metastases to the vas deferens (Figure 2) and testis (Figure 3) were found from Day 46. Moreover, only these groups showed ascites (Table I). Metastases to vas deferens and testis represent a process of remetastases since its tumoral behavior is different from the parental implanted groups. This is supported by the fact that metastatic cells, which have passed through a process of selectivity *in vivo*,

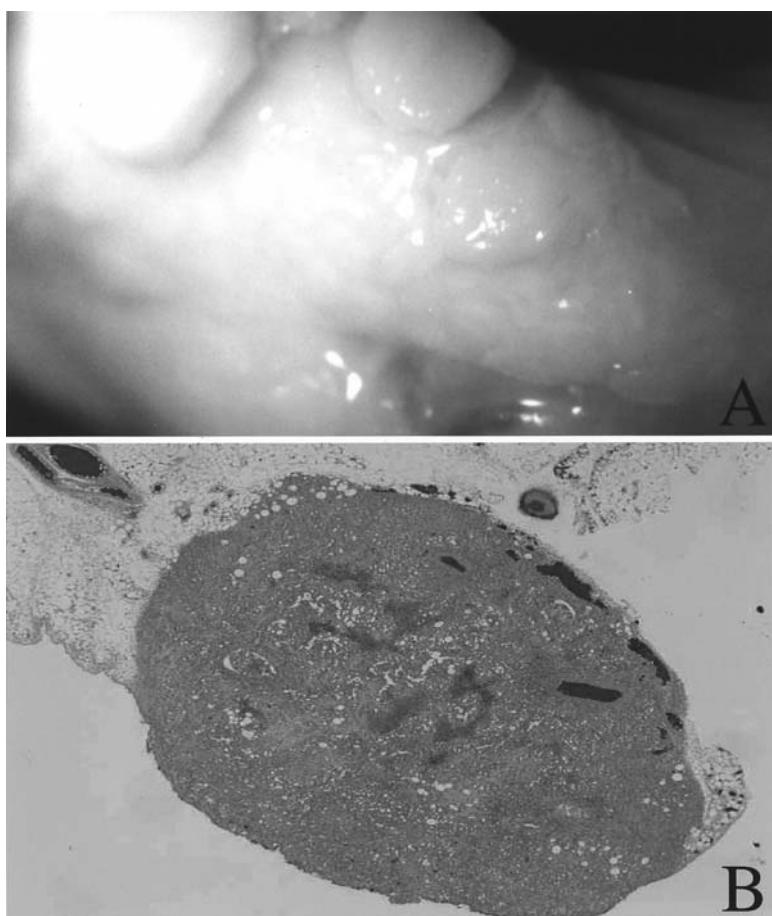


Figure 2. A case of LIM. (a) Multiple metastases to vas deferens. (b) Microscopic view of metastases showing a moderately-differentiated adenocarcinoma. HE, 40X.

may have a different behavior when compared with parental cells (21-23). In addition, established metastatic cells even when grown in the same "primary and seed organ", *i.e.*, pancreas, may show different metastatic behavior and different preference for "soil" organ (24).

This study suggests that the metastatic behavior of parental and metastatic cell lines is different. Thus, this should be considered in the planning of clinical or surgical treatment against pancreatic cancer. However, further studies will be necessary to clarify the mechanisms.

#### Acknowledgements

The authors would like to thank the Laboratory Animal Center and Molecular Genetics Center of Toyama Medical and Pharmaceutical University, Japan, Prof. Keiichi Yamamoto, Dr. Takashi Sakamoto, Dr. Hideki Arai, Dr. Renato Soares de Godoy, Dr. André Siqueira Matheus, Márcia Saldanha Kubrusly and Juliana Ikeda Niigaki for their support.

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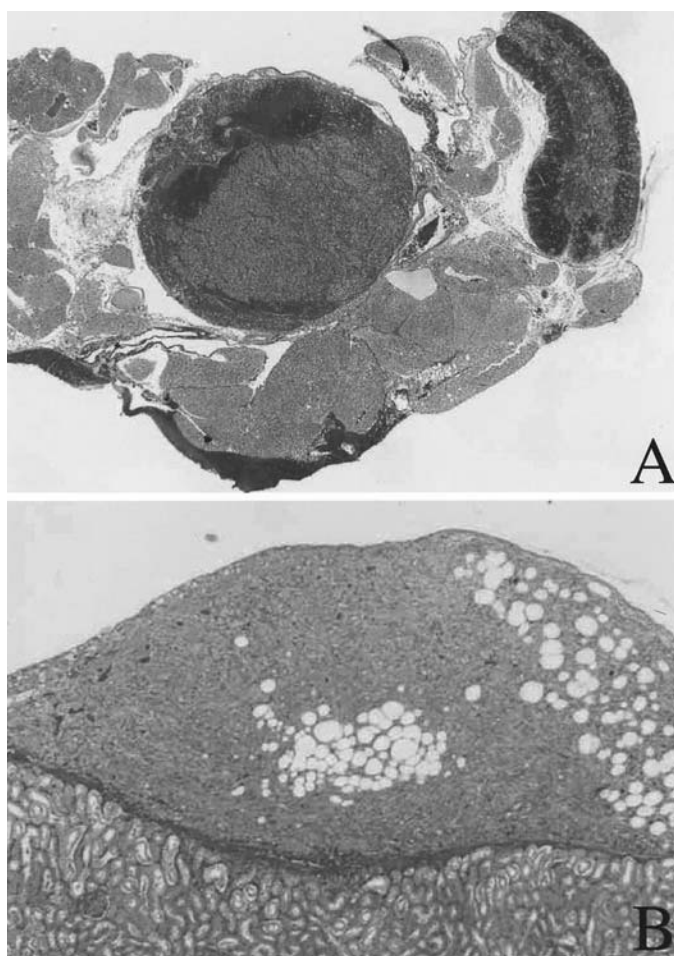


Figure 3. (a) A case of the LIM group with metastases to the testis (round organ) and to the epididymis (elongated organ). HE, 40X. (b) A case of PIH group with metastases to the kidneys. HE, 40X.

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Received September 29, 2003

Revised December 8, 2003

Accepted December 22, 2003