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Different Pattern of Metastasis in Liver Implanted Pancreatic Cancer between Young and Old Syrian Golden Hamsters

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Abstract. *We have previously reported on the "return trip" metastases from the liver to the pancreas in a hamster experimental pancreatic cancer model. Because the pancreas is the main metastatic site of liver-implanted pancreatic tumors, our aim was to clarify whether the metastatic sites differ in young and old tumor-bearing animals. HaP-T1, a continuous tissue-cultured cell line, derived from BHP-induced pancreatic adenocarcinoma, was implanted into the liver. The animals were divided into two groups: A) younger than 26 weeks and B) older than 26 weeks. Three animals from each group were sacrificed on Days 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98, to study the metastatic sites. Survival was also studied. After death, necropsy was performed. Resected and necropsied specimens were analyzed histopathologically and by PCR/RFLP analysis to confirm the presence of K-ras point mutation. The success rate of implantation was 100%. Survival was 102.3±2.5 days in group A and 95.3±1.5 days in group B. Animals of group A, sacrificed weekly until Day 70, showed metastases only to the pancreas ("return trip"), while this phenomenum happened only in animals sacrificed on Day 35 in group B. In group A, on Days 77, 84, 91 and 98, metastases were also found in the kidneys, lymph nodes, ovary and testis. In hamsters of group B, metastases were found in multiple sites such as the pancreas, vas deferens, ovary and testis ("multiple journeys"). All intra-hepatically-implanted tumor and metastatic sites showed the K-ras point mutation. This homologous implantation model may be helpful for further research into the process of metastasis and its relationship with the immunological response.*

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Pancreatic cancer continues to represent a medical challenge (1-3). Although nude mice have been widely used as *in vivo* models, they are not completely immuno-suppressed because their T-cell function is not absent, just decreased (4). As these animals get older, the increase of T-cells may induce false-positive results (5-8). On the other hand, homologous models, such as hamsters, have the advantage that the T- and B- cells are preserved. Moreover, the immunological response decreases as the animal gets older, similar to humans (9, 10). We have previously reported on the "*return trip*" metastasis from the liver to the pancreas in a hamster experimental pancreatic cancer model (11). Therefore, because the pancreas is the main metastatic site of liver-implanted pancreatic tumors, our aim was to clarify whether the metastatic sites differ in young and old tumor-bearing animals.

Materials and Methods

Cell line. HaP-T1, a continuous tissue cultured cell line, derived from BHP-induced pancreatic adenocarcinoma, was used for these experiments (12). This cell line shows a mutation from GGT to GAT in codon 12 of the K-*ras* gene (13).

Animals. Syrian golden hamsters (GN strain) of both sexes, aged from 16 to 42 weeks, weighing on average 132 g, were used. They were maintained in the Animal Center of Toyama Medical and Pharmaceutical University, Japan, under 12/12 light/dark and given chow and water *ad libitum*.

Experimental design. The animals were divided into 2 groups: A) younger than 26 weeks and B) older than 26 weeks. The tumor graft was implanted into the liver. Follow-up was done by abdominal palpation and body weight evaluation. Three animals of each group were sacrificed on Days 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98, to study the metastatic sites. The animals were followed-up. The survival time was studied in 3 hamsters of each group. After death, necropsy was performed. Resected and necropsied specimens were analyzed histopathologically and by PCR/RFLP analysis to confirm the presence of the K-*ras* point mutation.

Table I. *Comparison between Group A and Group B.*

	Group A (<26 weeks)	Group B (> 26 weeks)
Success of implantation	100%	100%
Survival time (days)	102.3±2.5	95.3±1.5
Metastatic sites		
Day 35	Pa	Pa
Day 42	Pa	Pa, VD
Day 49	Pa	Pa, VD, Te
Day 56	Pa	Pa, VD, Te/Ov[1]
Day 63	Pa	Pa, VD, Te/Ov[1]
Day 70	Pa	Pa, VD, Te/Ov[1], Ki
Day 77	Pa, Ki	Pa, VD, Te/Ov[1], Ki, Ln
Day 84	Pa, Ln	Pa, VD, Te/Ov[1], Ki, Ln, As
Day 91	Pa, VD	Pa, VD, Te/Ov[1], Ki, Ln, As
Day 98	Pa, VD, Te/Ov[1]	-

N=3 in each group. There were no intra-hepatic metastases. Pa, pancreas; VD, vas deferens; Te, testis; Ov, ovary; Ki, kidney; Ln, lymph node; As, ascites; Te/Ov[1] depending on the sex of the sacrificed animal.

Preparation of tumor cell suspensions. Tumor cell suspensions (2 x 10^6 cells/ml) were inoculated subcutaneously. One month later, the growing tumor was resected for implantation.

Liver implantation. The animals were anesthetized with ether inhalation and sodium pentobarbital (5 mg/kg/body weight) intraperitoneally and placed in a sterile field. A right subcostal incision was made and one piece of tumor was implanted in the frontal lobe of the liver after making a hole with a scalpel. The hole was closed with Vycril® 7.0 sutures (Ethicon, NJ, USA). The abdominal wall was closed in two layers with nylon 4.0 sutures (11).

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

Detection of K-ras point mutation. DNA was extracted with sodium dodecyl sulfate and proteinase K. The crude extract was purified using the phenol-chloroform-isoamylalcohol precipitation method (14). The PCRs were performed in 50 μl-reaction volume, as described previously (13, 15, 16). DNA was amplified for 35 cycles of 92˚C for 20 sec; annealing, 50˚C for 2 min; extension, 72˚C for 20 sec. The primers used were as follows: sense 5'-ACT GAA TAT AAA CTT GTG GTA GTT GGA CCT-3' (13,15-17) and antisense 3'-TTA TAC TAG GAT GCT ATC TC-5'(13,15,16,18). PCR fragments were separated on 2% agarose gels. Sixteen μl of PCR products was digested with 10 U of Bst N-I (New England Biolabs®, Inc., MA, USA) at 60˚C for 3 h, in a total volume of 50 μl.

Results and Discussion

The success rate of implantation was 100%. The survival time was 102.3±2.5 days in group A and 95.3±1.5 days in group B. Animals of group A, sacrificed weekly until Day 70, showed metastases only to the pancreas ("*return trip*"), while this phenomenum happened only in animals sacrificed on Day 35 in group B. In group A, on Days 77, 84, 91 and 98, metastases were also found in the kidneys, lymph nodes, ovary and testis. In hamsters of group B, metastases were found in multiple sites such as the pancreas, vas deferens, ovary and testis ("*multiple journeys*"). Moreover, after Day 70, lymph node, kidney and peritoneal dissemination with ascites were found in this group (Table I). All intra-hepatically-implanted tumor and metastatic sites showed the K-*ras* point mutation (Figure 1).

In our previous report of "*return trip*" metastases, the pancreas was the only target metastatic organ (12). In an orthotopic implantation model, the metastatic sites were similar to those described in the present study. Thus, the spontaneous metastases that occurred in the present model may be a consequence of both the "*seed and soil*" hypothesis and the mechanical hypothesis, which may be facilitated by the aging process. Nevertheless, the present homologous model is better than nude mice models, because the animals can be observed for a long period and tumoral behavior with the process of metastasis better analyzed. Moreover, decreased immunological response related to aging may play a role in this process.

Macieira-Coelho *et al.* (19) reported that age-dependent modifications of extracelullar matrix (ECM) biosynthesis and degradation may occur. Moreover, they stated that the neoplastic process exhibits well characterized age-dependent variations. Labat-Robert (20) showed age-dependent modifications of tissue and plasma fibronectin, which interfere in the development of cancer.

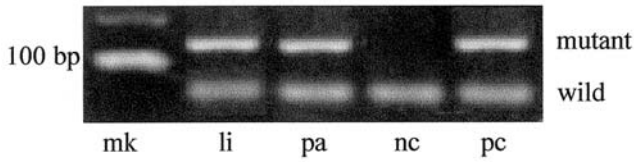

Figure 1. *PCR/RFLP analysis. Ethidium bromide-stained gel. mk: marker, li: liver, pa: pancreas, nc: negative control (DNA extracted from the liver of a 12-week-old hamster), pc: positive control (DNA extracted from HaPT1 cell line). Cancer samples show two bands, mutant and wild.*

Invasion and metastasis may be facilitated by proteins, which stimulate tumor cell attachment to host cellular or ECM determinants, tumor cell proteolysis of host barriers, such as the basement membrane, tumor cell locomotion and tumor cell colony formation in the target organ of metastasis.

Most steps of the metastatic cascade may be enhanced when the host immunological function is suppressed. We also believe that vascular changes may facilitate metastasis.

In addition, the presence of the K-*ras* point mutation in all implanted tumors and metastatic sites showed that they were really pancreatic adenocarcinomas, so these animals may develop spontaneous tumors when they get old.

In conclusion, this homologous implantation model may be helpful for further research related to the process of metastasis and its relationship with the immunological response. Moreover, it may also be useful for the study of tumor behavior.

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