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Radioimmunoscintigraphy of human pancreatic carcinoma xenografts in nude mice with ^{131}I-labeled monoclonal antibody

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Encouraged by reports of radioimmunoimaging of colorectal carcinomas[1–3] and by examining an immunohistochemical report on resected pancreas cancer tissues[4], we studied the diagnostic potential of radioimmunoimaging with the radioiodinelabeled monoclonal antibody to the surface antigen of a pancreas cancer cell line. A monoclonal antibody (MoAb; HC-1) to a human pancreas cancer cell line (HGC25)[5] was labeled with radioiodine and injected into athymic nude mice implanted with human pancreas cancer cells. Antibody HC-1 was cleared from the circulation and accumulated significantly in the implanted tumor sites.

Key words: radioimmunoimaging, pancreas cancer, monoclonal antibody

INTRODUCTION

THE DETECTION of malignant tumors by scintigraphy has become a theme of human cancer research. The development of the hybridoma technology which has allowed production of monospecific antibodies has followed the improvement of this research technique.[6] A monoclonal antibody (MoAb; HC-1) to a human pancreatic carcinoma cell line (HGC25) was established previously.[4] Briefly, this antibody (HC-1) is an IgG2a which reacts not only with pancreas cancer cell lines but also with other cancer cell lines such as colon, stomach cancer and so on. And it does not detect HLA associated antigens since it failed to react with human myeloid and lymphoid cell lines and normal hematopoietic cells. Immunohistochemical analysis showed that this monoclonal antibody reacted with pancreas cancer tissues, but did not react with normal and other malignant epithelial tissues. This report describes the localization of human pancreatic carcinoma xenograft in nude mice using ^{131}I-labeled HC-1.

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MATERIALS AND METHODS

Antibody preparation: The monoclonal antibody HC-1 whose biological characterization was reported previously by Usui and Koshiba was isolated from ascitic fluid obtained from hybridoma bearing pristine primed BALB/c mice and purified by DEAE chromatography (0.04 M phosphate buffer, 0.03 M NaCl, pH 8.0) and then by Protain A affinity chromatography (0.1 M citrate-phosphate buffer, pH 4.5).[7] Radioiodination of whole IgG with iodine-131 (^{131}I) was performed by the chloramine-T method.[8] Unbound iodine was removed by gel-filtration on a Sephadex G-50 column (PBS, pH 7.5). The result showed a specific activity of 2.5 μCi/μg.

Tumor preparation: 0.1 m*l* (1×10^6/m*l* in PBS of the cloned pancreatic cancer cell line (HGC25)) was inoculated subcutaneously into the back or abdomen of BALB/c athymic nu/nu mice. The tumors which grew to 1.0–1.5 cm in diameter at 3–4 weeks after the inoculation were used in this study. The administration of non-radioactive iodine to the mice started on the seventh day before the injection of radioiodinated antibody and continued throughout the experiments.

Biodistribution studies and radioimmunoimaging: For the scintigraphic examination and the biodistribution study, radioiodinelabeled antibodies at

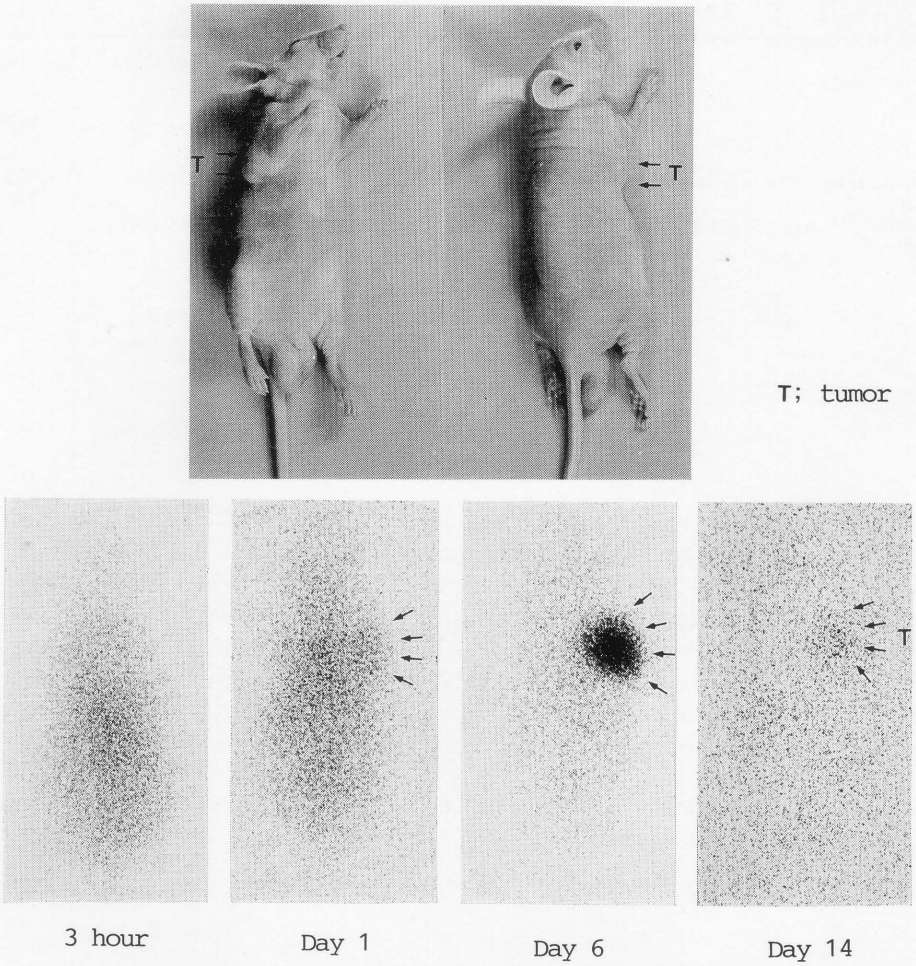

Fig. 1 Scintigrams (postero anterior view) of a mouse bearing HGC25 tumor, taken at 3 hours, and 1, 6 and 14 days after intravenous injection of I-131 labeled whole IgG (HCl). The site of the tumor is shown photographically.

doses of 10 to 20 μg (0.925 to 1.85 MBq (25 to 50 μCi)) were injected into the tail vein of nude mice bearing the tumor.
figures).

Scintigrams were obtained with a gamma camera equipped with a pinhole collimator. The tumors and the selected organs from the mice sacrificed at suitable times were removed, weighed and counted. Biodistribution data were expressed as a percentage of the injected dose per gram of tissue normalized to a 20 g mouse. The tumor to blood ratio was also calculated.

RESULTS

^{131}I-labeled MoAb HC-1 imaging: Scintigrams were obtained at 3 hours, 1 day, 6 days and 14 days after injection of the ^{131}I labeled monoclonal antibody. It took 6 days to decrease the background radioactivity so that the tumor was well defined (Fig. 1). On the other hand, no significant activity in the transplanted tumors was observed in the scintigrams after intravenous injection of free ^{131}I (not shown in figures).

Biodistribution studies of labeled antibodies: The results of the *in vivo* localization of the labeled antibodies are shown in Table 1, whose figures indicate the percentages of injected dose per gram. The tumor-to-blood ratio of the radioiodinated whole IgG (HC-1) progressively increased with time, and the radioactivity of the transplanted tumors is high enough to separate from liver.

DISCUSSION

Hybridoma technology has provided numerous murine monoclonal antibodies specific for human malignant tumors. Various degrees of success have been obtained in studies of tumor localization using labeled MoAbs that react with known tumor makers such as carcinoembryonic antigens[9–10] and alpha-fetoprotein[11] and MoAb which react with specific

Table 1 Biodistribution of radioiodine labeled whole IgG of HC-1 in tumor bearing nude mice

Organ	%dose/g (mean and range)	
	Day 2 (N=4)	Day 8 (N=2)
Blood	5.29 (2.36–9.21)	3.19 (2.90–3.48)
Brain	0.26 (0.13–0.44)	0.06 (0.06–0.06)
Bone	1.45 (0.92–2.31)	0.32 (0.32–0.32)
Muscle	1.63 (0.27–3.43)	0.18 (0.17–0.19)
Lung	4.82 (2.78–5.75)	1.44 (1.19–1.70)
Stomach	1.77 (0.99–3.11)	0.23 (0.16–0.30)
Intestine	1.06 (0.63–1.68)	0.21 (0.18–0.24)
Liver	1.38 (0.92–2.31)	0.45 (0.42–0.48)
Spleen	3.03 (1.64–5.58)	0.81 (0.76–0.86)
Kidney	4.24 (2.46–6.43)	0.97 (0.91–1.02)
Tumor	9.16 (7.46–11.4)	9.89 (8.88–10.9)

*Tumor/Blood: 1.73 3.10 (mean)
*Tumor/Liver: 6.64 22.0 (mean)

surface antigens of tumors including breast, colon, and other cancers.[12]

Some monoclonal antibodies to pancreatic carcinoma have been produced,[13,14] but there have been only a few reports on radioimmunoscintigraphy.[15,16] We demonstrated here the specific radioimmunolocalization of xenografts of human pancreas carcinoma in nude mice by ^{131}I labeled whole IgG (MoAb; HC-1). The biological characteristics of HC-1 were previously reported in detail.[4] As mentioned in the introductory part of this article, MoAb HC-1 is an IgG2a by which the antigenic determinant is recognized, and is a glycoprotein with a molecular weight of 130 k Daltons found in human pancreatic carcinoma cells but not in tumor extracts.

For clinical use, ^{111}In has many advantages over ^{131}I, because of the low radiation dose and suitable energy for scintigraphic imaging. Moreover, the labeling of antibodies can be done rapidly and efficiently through chelation with DTPA.[9,11] As a matter of fact, ^{111}In labeled monoclonal antibodies have been clinically used in imaging of the malignant melanoma and gastrointestinal tumors. However, non specific high tracer accumulation in normal organs, such as the liver, kidneys and spleen is observed, and this may interfere with the detection of abdominal tumors. It seems that the diagnostic value is limited by radioimmunoimaging with ^{111}In conjugated antibody for pancreas cancer and for malignant tumors in the upper and lower abdomen.

The final goal of this study is to examine and treat the patient with pancreas cancer, so we made mention of *in vivo* localization of the radioiodinated monoclonal antibody (HC-1). In serial scintigrams, excellent images of the tumor were obtained 6 days after the injection of ^{131}I-IgG (HC-1), and the radioiodine activity in liver, kidneys and spleen was low. The investigation of *in vivo* localization of the radiolabeled antibody to the membrane associated antigen of one pancreatic carcinoma cell line (HGC25) of pancreatic carcinoma is summarized above. Because the problem has yet to be solved, we could not draw a conclusion on the heterogeniety and the antigenic modulation of pancreas tumor cells in this radioimmunoimaging study. It is necessary to continue the investigation of these problems. The present examination is still at the animal experimental stage.

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