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Detection of Locally Recurrent Colorectal Cancer with Radiolabeled Monoclonal Antibody H-15

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H-15 (HT-29-15) is an IgG1 mouse monoclonal antibody (mAb) to a cell surface antigen (molecular mass, 200,000 daltons) present on virtually all colorectal cancers and also in normal pancreatic ducts and bile ducts, but not in other normal tissues. The biological distribution and imaging characteristics of iodine-131 (^{131}I)-labeled mAb H-15 were studied in 5 primary colorectal cancer patients and 9 patients with local recurrence of colorectal cancer. H-15 mAb labeled with 0.5–10 mCi of ^{131}I was administered 7 to 8 days before surgery at 4 dose levels, ranging from 0.2 to 6 mg. Selective mAb H-15 localization to tumor tissues was demonstrated in 6 of 12 patients with antigen-positive tumors: in two patients, recurrent tumors were negative to H-15 mAb, although the primary tumors were positive. In six patients with positive radioimaging, tumor:normal tissue ratios ranged from 2.05 to 5.35 and tumor:serum ratios from 1.18 to 2.73. The clarity of images seems to correlate well with the latter ratios. Technetium-99 (^{99m}Tc)-albumin blood pool studies in selected cases showed that local recurrence of colorectal cancers was hypovascular, emphasizing the selective localization of mAb H-15 despite poor blood flow distribution in the tumors. The results altogether demonstrated that radioimmunodetection with ^{131}I mAb H-15 is valuable for differentiating recurrent colorectal cancer from granuloma formation after surgery.

Key words: Colon cancer — Radioimmunodetection — Monoclonal antibody

Despite modern surgical technology, the prognosis of gastrointestinal cancers is still poor. In patients with advanced cancers, even in cases where complete curative resection seems to have been successfully performed, metastasis to the liver, peritoneal dissemination or local recurrence is likely to appear in a significant number of cases. The use of monoclonal antibodies (mAb[8]) as a qualitative diagnostic tool and eventually as targeting agents for therapy has attracted much attention despite the many technical challenges that confront its clinical application.[1–4] Several limitations in the use of mAbs for tumor imaging and therapy have already been discussed by various investigators; i.e., selective delivery of only a small amount of mAbs to the tumor site,[5–11] influence of circulating antigens in the blood stream[5, 12–15] and heterogeneity of antigen expression in tumors.[9, 13, 16, 17]

In imaging studies of human colorectal cancer, several antigenic systems have been studied in order to search for correlations between external imaging and biopsy-based measurements of tissue uptake of antibody, i.e., carcinoembryonic antigen (CEA),[5, 7, 9, 10] sialylated Tn (TAG72.3),[15, 16] A-33,[18] sialylated Le^a,[19, 20] H-15 (HT-29-15),[11] 791T/36[21] and 17-1A.[22, 23] These studies were performed mostly in cases with liver metastasis of colorectal cancer, and the tumor : normal tissue (mostly normal liver) ratios were determined. Antibody localization was shown to be dependent on the expression of the corresponding antigen on colon cancer,[9, 15, 16] but not all antigen-positive tumors were imaged, suggesting possible involvement of additional factors in antibody localization depending on the types of tumor cells and/or the characteristics of each individual patient tested.

In this study, for the detection of local recurrence of colorectal cancer, we utilized the mAb H-15 (HT-29-15) formerly used in the radioimmunodetection of liver metastasis of colon cancer.[11, 24] The reason for selecting such local recurrence as a target of mAb H-15 radioimmunodetection is based on the clinical necessity to differentiate tumor recurrence from granuloma formation after surgery.[25, 26] Qualitative diagnosis by mAb imaging should be of great help to determine the need for extensive secondary surgery, including pelvic exenteration.

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[8] Abbreviations: mAb, monoclonal antibody; I, iodine; Tc, technetium; CEA, carcinoembryonic antigen; Ig, immunoglobulin; MDP, methylene diphosphonate; CT, computed tomography; HAMA, human anti-mouse Ig antibody; A-P, anterior posterior; P-A, posterior anterior.

Furthermore, information obtained by such studies will be useful for exploitation of the mAbs as therapeutic agents.

MATERIALS AND METHODS

Production and fractionation of mAb H-15 H-15 (HT-29-15) hybridoma cells[24] were grown as ascites in *nu/nu* mice. Batches of ascites with high antibody titer were pooled for purification and ultracentrifuged at 100,000*g* to remove cell debris and lipoproteins. The immunoglobulin (Ig) fraction was precipitated with 0% to 50% ammonium sulfate, redissolved in 0.03 mol/liter HEPES, pH 8.0, then chromatographed over Sephadex G-50 superfine (Pharmacia-LKB, Uppsala, Sweden) and the antibody eluted was passed through an S Sepharose fast-flow column for cation exchange chromatography (Pharmacia-LKB). The antibody was then equilibrated in phosphate-buffered saline (pH 7.0) by passage through a Sephadex G-50 superfine column, followed by sterile filtration. All procedures were carried out aseptically with pyrogen-free materials. Purity was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by silver staining. The titer of H-15 antibody was assessed throughout the procedure by means of a mixed hemadsorption assay with SW-1083 colon cancer cell line as target cells. The lot of H-15 mAb thus obtained met the guidelines for patients' use of the Food and Drug Administration, USA, including tests for murine viruses, rabbit pyrogenicity, *Limulus* assay for endotoxin, bacteria and mycoplasma contamination, and mouse and guinea pig toxicity.

Radioiodination of mAb H-15 Doses of 0.2, 2.0, 4.0, or 6.0 mg of mAb H-15 (maximal volume, 2.4 ml) were labeled with 0.5 to 10 mCi of ^{131}I sodium iodide (Japan Mediphysics, Chiba) using the chloramine T method.[27] The reaction was terminated with sodium metabisulfite, and the product was passed over a sterile Sephadex G-25 fine column (Pharmacia-LKB). Fractions with peak radioactivity were pooled and passed through a 0.2 μm filter. The freshly prepared sample was tested for binding of ^{131}I to mAb H-15 using trichloroacetic acid precipitation (>95% counts bound) and for immunoreactivity using a mixed hemadsorption assay. Immunoreactivity of ^{131}I-labeled mAb H-15 as estimated by this method was 50 to 80%.

Patient selection Patients in this study had histologically proven colorectal carcinoma. Each patient was scheduled for one of the following three surgical procedures; resection of primary tumor with regional lymph node dissection, biopsy of the specimen in unresectable cases or pelvic exenteration for local recurrence of the tumor. The performance status of patients was 0 or 1. Eligibility criteria included serum creatinine less than 1.5 mg/100 ml, bilirubin less than 2 mg/100 ml, granulocytes more than 2000/μl, and prothrombin time less than $\times$1.5 control. Patients with serious cardiac, pulmonary or infectious disease prior to the antibody injection, or an expected survival of less than three months were excluded. Informed consent was obtained from all patients before participation in the study. This study and consent forms were approved by the ethical committee and by the institutional review board of Aichi Cancer Center.

Administration of ^{131}I-labeled mAb H-15 Beginning at 72 h prior to and for 7 days after antibody administration, patients were treated orally with 1 ml of a saturated solution of potassium iodide daily. An intradermal skin test with 0.1 μg of mAb H-15 was performed 48 h prior to antibody administration to detect immediate and delayed-type hypersensitivity reactions. No positive skin test reaction was observed among the patients studied. ^{131}I-Labeled H-15 was administered intravenously in a physiological saline solution containing 5% human serum albumin (total volume 100 ml) through a 0.2 μm filter over a period of 1 h. Dose levels of the antibody were 0.2 mg (2 cases), 1 mg (1 case), 2 mg (1 case), 4 mg (5 cases) and 6 mg (5 cases). The range of radioactivity administered in these groups of patients was 0.5 to 10 mCi.

Radioimaging, and tissue, blood and urine samples ^{131}I anterior (A) and posterior (P) body images (Hitachi gamma camera, Tokyo) were obtained 1 h after antibody administration and then on days 1, 4 and 6. Total urine output was collected each day for 7 days or at least until surgery to monitor isotope excretion. To determine radiolabeled antibody levels, blood was drawn at 1, 2, 4, and 8 h after antibody administration and then on days 2, 4 and 6. In addition, ^{99m}Tc-methylene disphosphonate (MDP) scans, arteriograms, computed tomography (CT) scans, ultrasonic examinations and chest X-rays were obtained. Blood samples were drawn during surgery to compare radiolabeled antibody levels in the blood with those in the biopsy material. Just after surgery, specimens of the tumor site and the normal colonic epithelium were obtained to evaluate the distribution of radiolabeled H-15 antibody. A portion of each specimen was used for routine histologic evaluation and for detection of H-15 antigen using the immunoperoxidase procedure. Enzyme-linked immunosorbent assay (ELISA) was used to measure anti-mouse Ig antibody in patients' sera.

RESULTS

Characteristics of patients Fourteen patients were enrolled, and all of them were assessable for antibody localization by the range of parameters used in this study (Table I). All patients had colonic or rectal adenocarcinomas confirmed by histology. All patients had well or

Table I. Patients' Characteristics

Patient No.	Age	Sex	Primary site	Imaging site	Tumor size (cm in diameter)	Surgical procedure[a)]
1	66	M	Rectum	Primary + lymph node	4.6	AP
2	43	F	Rectum	Primary + lymph node	4.4	AP
3	40	F	Colon	Lymph node	3.5	Sigmoidectomy
4	66	M	Rectum	Primary + peritoneal metastasis	5.4	LAR
5	72	M	Rectum	Local recurrence	2.8	PE
6	68	F	Rectum	Local recurrence	3.8	PE
7	72	M	Rectum	Primary + lymph node	5.5	AP
8	59	F	Rectum	Local recurrence	8.9	PE
9	42	F	Rectum	Local recurrence	3.5	LAR
10	79	F	Rectum	Local recurrence	3.0	PE
11	54	F	Rectum	Local recurrence	4.8	PE
12	62	M	Rectum	Local recurrence[b)]	6.0	PE
13	73	M	Rectum	Local recurrence[b)]	3.7	PE
14	50	M	Rectum	Local recurrence	4.5	PE

a) AP, abdominoperineal resection; LAR, low anterior resection of rectum; PE, pelvic exenteration.
b) Recurrent tumors were not reactive with mAb H-15, although primary tumors were positive.

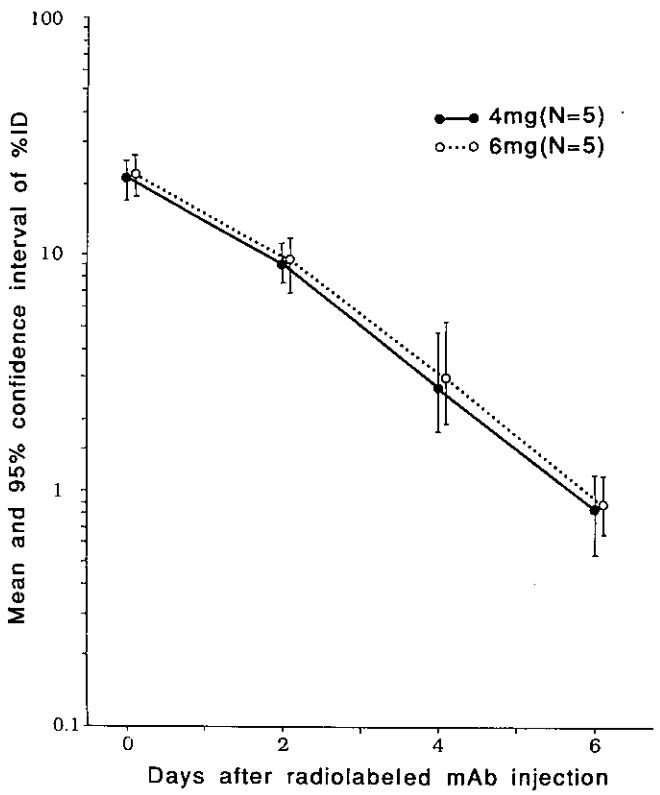

Fig. 1. Pharmacokinetics of ^{131}I-mAb H-15. ^{131}I levels are indicated by the mean and the 95% confidence interval of percentage of the injected dose (% ID) per liter of serum collected until day 6. Mean values for two dose levels of mAb H-15: 4 mg (solid line) and 6 mg (dotted line).

moderately well differentiated tumors with variable preoperative CEA and CA19.9 levels. One patient had primary sigmoid colon cancer and metastasis to the paraaortic lymph nodes. The other 13 patients had primary cancer in the rectum: nine had local recurrence of the tumor in the pelvis, while three had obvious lymph node metastasis and one had peritoneal dissemination of the tumor. Prior to antibody administration, biopsy specimens of the primary tumor or tissue specimens taken at the previous operation in the case of recurrent tumor were proved to be positive for H-15 mAb by immunohistological studies. In patients No. 12 and No. 13, however, immunostaining with H-15 mAb was negative in the resected recurrent tumors, presumably due to the growth of antigen-negative tumor clones.

Toxicity and development of human anti-mouse Ig antibody (HAMA) ^{131}I-Labeled H-15 antibody was infused over one hour at a rate of 0.2 to 6 mg/h. No immediate toxicity was observed. In case No. 13, fever up to 38.0°C was noticed 3 h after the administration, but it promptly disappeared after the injection of antihistamines.

HAMA became detectable by ELISA as early as the day of operation in three patients, and all patients developed HAMA by day 30. From days 5 to 8, the predominant HAMA was IgM, whereas by day 30, IgG was also detected, together with IgM antibodies.

Pharmacokinetics of ^{131}I-labeled mAb H-15 ^{131}I-Labeled mAb H-15 was administered to the patients at five dose levels (0.2, 1, 2, 4 and 6 mg). At the end of the 1 h infusion, the mean serum level was 21% of the injected dose per liter of serum, suggesting little immediate diffusion of mAb H-15 into the extravascular space or absorp-

Table II. Distribution of Radiolabeled mAb H-15 in Patients with Metastatic Colon Cancer

Patient No.	No. of days from injection to operation	% Injected dose ($\times 10^3$) per gram of tumor tissue	Dose of mAb H-15 (mg)	Tumor:nontumor ratio	Tumor:serum ratio	Imaging
1	3	1.8	0.2	1.00	0.26	(−)
2	3	3.4	0.2	1.77	0.33	(−)
3	5	0.4	1.0	4.00	2.73	(+)
4	7	3.4	2.0	1.57	0.83	(−)
5	17	0.1	4.0	1.00	0.15	(−)
6	5	2.3	4.0	5.35	1.18	(+)
7	7	2.2	4.0	1.22	0.65	(−)
8	5	2.7	4.0	4.50	1.58	(+)
9	7	3.5	4.0	3.40	1.08	(−)
10	6	2.4	6.0	2.16	1.44	(+)
11	6	2.9	6.0	3.42	1.78	(+)
12[a)]	7	1.1	6.0	0.95	0.47	(−)
13[a)]	6	0.7	6.0	1.08	0.54	(−)
14	14	1.4	6.0	2.05	1.28	(+)

a) Recurrent tumors were not reactive with mAb H-15, although primary tumors were positive.

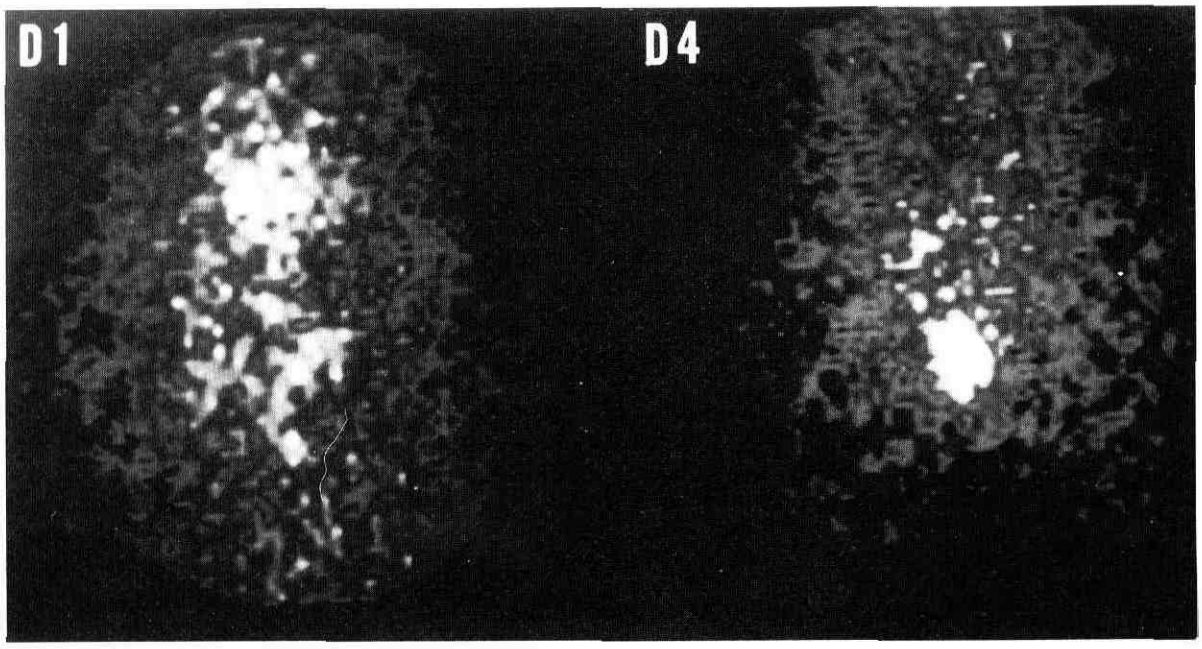

Fig. 2. Serial lower abdominal body images of patient No. 6 following administration of 4 mg of ^{131}I-mAb H-15. Initial scan on day 1 (D1) (left) shows vascular ^{131}I-mAb H-15 distribution. Day 4 (D4) localization to locally recurrent rectal cancer (right) is visualized due to blood pool ^{131}I-mAb H-15 clearance.

tion by circulating H-15 antigen. A mixed hemadsorption test performed at the end of infusion was not able to detect the specific H-15 reactivity in the serum, probably because of the dilution of the mAb in the serum.

Clearance of ^{131}I-mAb H-15 from the blood showed a half life (t1/2) α of 7.2 h and a (t1/2) β of 40.5 h. No significant differences in clearance rates were observed at two dose levels, 4 mg and 6 mg (Fig. 1).

Radioimaging study and distribution analysis Serial abdominal images were obtained from day 1 after antibody infusion and then on days 4 and 6, or until surgery. Specific antibody localization was assessed by correlating

mAb scans with (a) CT scans, (b) tumor site recorded at surgery, (c) histology of the resected specimen, and (d) radioactivity measurements in resected or biopsied normal and malignant tissues (Table II). Imaging revealed the presence of tumors in 6/14 patients. Since two patients were found to have H-15 antigen-negative tumors (patients No. 12 and No. 13), 6/12 antigen-positive tumors were demonstrated. The size of tumors ranged from 2.8 cm to 8.9 cm in diameter as determined by direct measurement during operation. There seemed to be no clear correlation between the tumor size and positive redioimaging in this study.

Typical radioimaging results are shown in Figs. 2 and 3. In Fig. 2, serial abdominal scans of patient No. 6 following administration of 4 mg of ^{131}I-mAb H-15 are illustrated. The initial image at 24 h after the injection was consistent with the blood pool. Signals to show local recurrence of the tumor became increasingly distinct with the clearance of mAb from the blood pool, and by day 6, the recurrent tumor was clearly distinguishable from the surrounding pelvic tissues and organs. In Fig. 3, A-P and P-A views of patient No. 10 are presented. In the patients with positive imaging, the P-A image was stronger than the A-P image, implying the presence of radioactivity in the recurrent site, but not in the bladder. The tumor:normal colon ratio was 5.35 in patient No. 6, and *ex vivo* scans showed clear radioactivity at the tumor site in the resected specimen (Fig. 4). In general, the clarity of images obtained with external radioimaging correlated best with tumor:serum ratios, and to a lesser degree with tumor:normal colon ratios. Lesions showing tumor:serum ratios less than 1.00 were never visualized by external imaging, although some of them were visibe on *ex vivo* scans using the resected specimen. There was no apparent relation of the dose administered to the clarity of images, tumor:mormal colon ratios, tumor:serum ratios or the percent of injected dose of antibody taken up by the tumor.

mAb H-15 localization in relation to distribution of blood pool examined by ^{99m}Tc MDP scans The distribution of ^{131}I-labeled mAb H-15 and ^{99m}Tc-MDP in tumor, normal colon and serum was also tested in cases No. 8, 10 and 11 (Table III). In all cases, the tumor:normal colon ratio and tumor:serum ratio were over 1.00 for ^{131}I-mAb H-15, but ranged from 0.11 to 0.22 for ^{99m}Tc MDP, accounting for the specific accumulation of H-15 MoAb in tumor tissues. Whole abdominal images of patient No. 8 following administration of ^{99m}Tc-MPD (scan at one hour) and 4 mg of ^{131}I-mAb H-15 (scan on day 4) are presented in Fig 5. In the imaging of this patient, ^{99m}Tc-MPD scans showed a hypovascular area in the lower part of the pelvic region, suggesting the appearance of metastatic lesions as photopenic areas in the pelvis where abdomino-perineal resection of the rectum had been performed 1

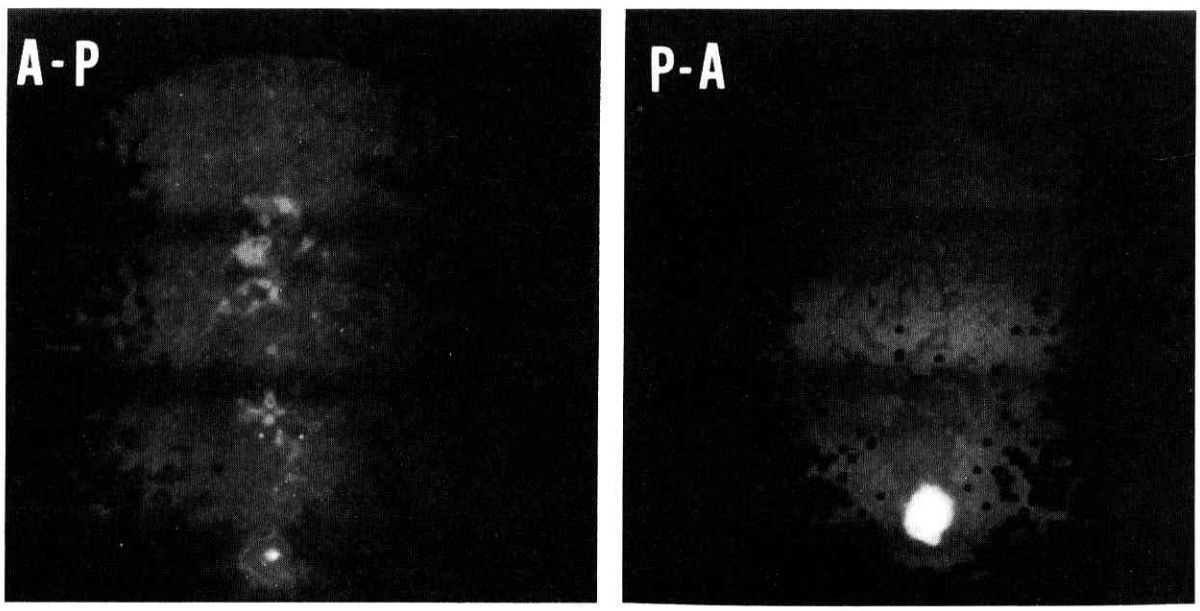

Fig. 3. Localization of mAb H-15 on day 6 to locally recurrent rectal cancer of patient No. 10. Simultaneous A-P (left) and P-A (right) scanning yielded a clearer image in the P-A view, indicating the presence of radioactivity in the posterior pelvic region.

year before radioimaging. Urostomy for urinary diversion was also performed in this patient before radioimaging and secondary surgery. On the other hand, recurrent tumor is clearly visualized as radiodense areas by the ^{131}I-mAb H-15 scans.

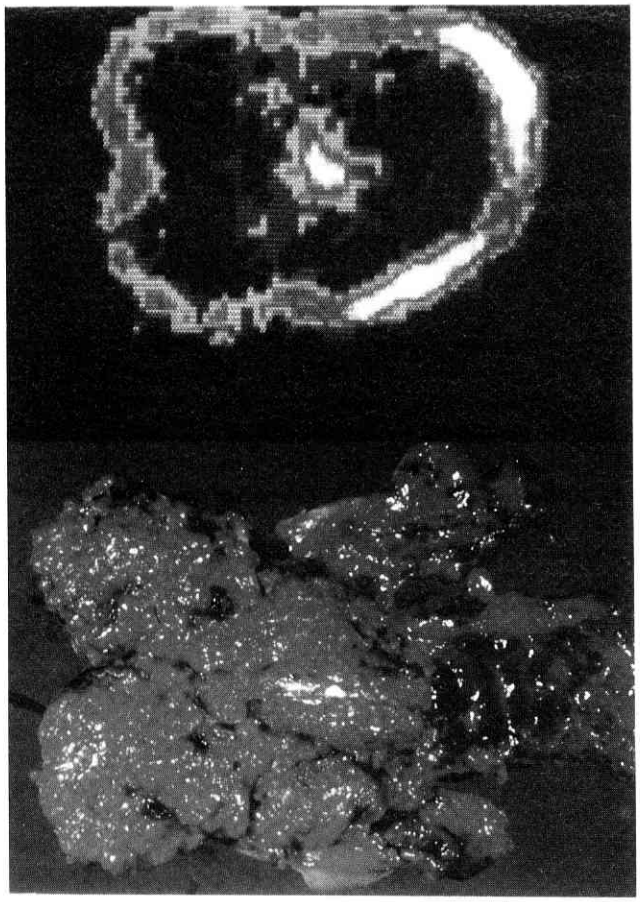

Fig. 4. Resected locally recurrent rectal carcinoma and surrounding normal tissue specimen from patient No. 6 following administration of ^{131}I-mAb H-15. *Ex vivo* scan shows distribution of ^{131}I-mAb H-15 to the tumor (upper). A tracer dose of ^{131}I is drawn surrounding the specimen to visualize the outline of the resected specimen. The lower photograph shows gross pathology of the specimen.

DISCUSSION

A number of tumor-imaging studies with mAb have been reported in recent years, but only a few of them have presented results on tissues.[7, 8, 9, 13] The present study with H-15 mAb imaging demonstrated a rather consistent and specific localization of recurrent or metastatic colorectal carcinoma. The patients studied were mainly those with local recurrence, and we concentrated on the imaging of the lower abdominal region. Furthermore, the consistency of the imaging site and the location of the tumor were confirmed by sequential (days 1, 4 and 6 after mAb injection) bidirectional A-P and P-A scanning. In all of our cases with positive radioimaging, the image of the mass in the pelvis was more intense and clearer in the P-A than in the A-P view, excluding the possibility that the imaging was of the urinary bladder. We always ensured that the bladder was empty by insertion of a urethral tube before the scanning.

Using resected or biopsied tissue specimens, the percentage of accumulation of mAb at the H-15 antigen-positive cancer tissues was evaluated, and it was found that the cases with the tumor:normal colon ratio over 2.0 amounted to 58% (7/12 cases), when antigen-negative tumor cases were excluded. This percentage is almost identical to the previous result with mAb H-15 (16 out of 21 cases) for imaging metastatic colon cancers to the liver.[11] In terms of radioimmunodetection of liver metastasis, however, this positive rate is not high, since when another mAb, A-33 was used, positive imaging was obtained in 19 out of 20 patients, and the tumor:liver ratio exceeded 10.0 in several patients.[18]

One of the advantages of using mAb H-15 for radioimmunodetection is the specific accumulation to the locally recurrent tumor mass in the pelvis, which is hardly accessible by conventional biopsy methodology. Specific accumulation at the tumor was also indirectly demonstrated by the simultaneous ^{99m}Tc-MPD scans and by the clear difference in tumor:blood pool ratio between these two radioisotopes. Unlike mAb A-33, which is cross-reactive with the normal colonic epithelium, mAb H-15 was negative with the normal colon, and so might yield more definite information about the tumor mass in the

Table III. Distribution of ^{131}I-mAb H-15 and ^{99m}Tc-MDP in Tumor Tissue, Normal Colon and Serum

Patient No.	^{131}I-mAb H-15		^{99m}Tc-MDP	
	Tumor:nontumor ratio	Tumor:serum ratio	Tumor:nontumor ratio	tumor:serum ratio
8	4.58	1.58	0.65	0.22
10	2.16	1.44	0.50	0.18
11	3.42	1.78	0.44	0.11

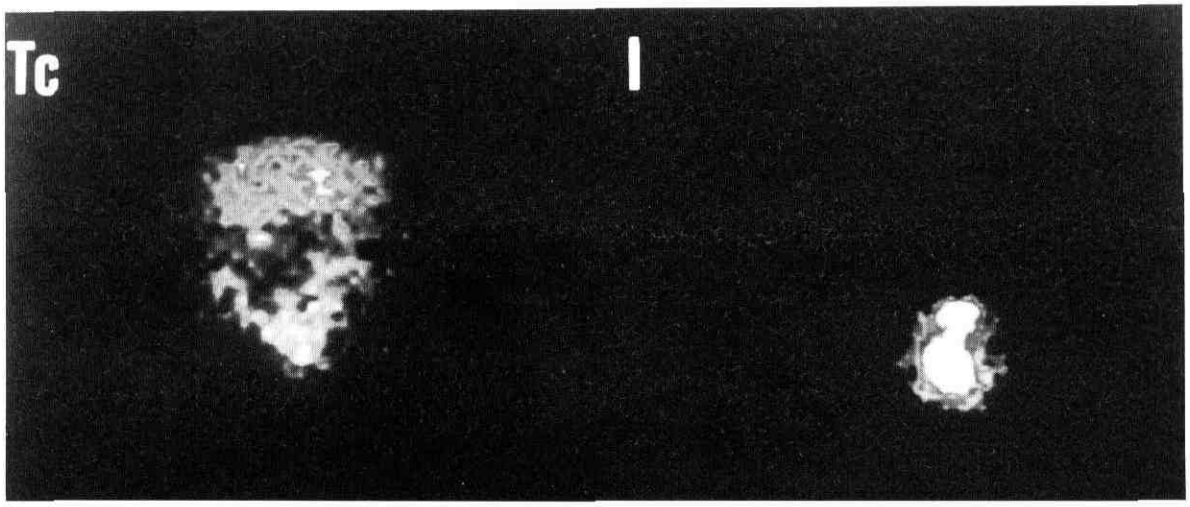

Fig. 5. Lower abdominal body images of patient No. 8 following administration of ^{99m}Tc-MPD and 4 mg of ^{131}I-H-15 mAb. ^{99m}Tc-MPC scans (scan at one hour; left) showed a hypovascular area in the lower abdomen. In contrast, a radiodense area consistent with the tumor mass is visualized by ^{131}I-H-15 mAb imaging (scan on day 4; right).

lower abdomen. In our study, positive tumor imaging was observed in 5 out of 9 locally recurrent rectal cancers (i.e. 5 out of 7 H-15 antigen-positive recurrent tumors), and the results as such provide a strong and objective indication for radical operation. At present, even by sophisticated CT or nuclear magnetic resonance (NMR) techniques, the qualitative diagnosis of pelvic mass after primary operation for colorectal cancer is quite limited. Accordingly, patients are sometimes subjected to an unnecessary extensive operation (e.g., pelvic exenteration) for benign granuloma mass detected by CT or NMR.[25, 26]

Despite these favorable findings with mAb H-15, there are still several points that need to be investigated. First, there were two H-15 antigen-negative tumors among 9 recurrent cancers, although the primary carcinoma tissues from the previous operation were positive with H-15 mAb. This kind of heterogeneity in antigen expression has been observed before, but it is hardly predictable, unless biopsy specimens are obtained from the recurrent tumors. In such cases, patients will receive an unfruitful administration of the mAb. The second problem is that we could not compare the distribution of mAb H-15 to that of a negative control mAb (which dose not react with H-15 antigen) of the same Ig isotype in this study. The use of ^{125}I-conjugated control mAb for standardization against ^{131}I-conjugated mAb H-15 was not approved by the ethical committee board of Aichi Cancer Center, because of the longer half life of ^{125}I which will increase internal exposure to the β emission. We did observe lower H-15 uptake in the two antigen-negative tumors, although this evidence is not conclusive. The third problem is that we have no data on radioimaging of patients with nonneoplastic mass, because our present study has been carried out with a relatively small number of patients. Needless to say, such patients should be studied in order to establish the clinical usefulness of mAb H-15 radioimaging. The fourth problem is the difficulty of evaluating the dehalogenation versus binding issue. Attempts were made by conventional immunohistochemical analysis to prove the binding of mAb H-15 to the resected specimens but without success, probably because the amount of the antibody molecules bound was below the sensitivity limit of our technique.

The eventual aim of using mAbs in clinical practice is the application of those mAbs for targeting therapy. Without accurate information on antibody localization and tissue specificity analysis in patients, use of these mAbs as a therapeutic agent would be potentially harmful to the patients. In this regard, establishing the specificity of the antibody is the most important task for investigators who are involved in radioimmunodetection studies.

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