Radioimmunolocalization of human colorectal carcinoma xenografts with the F(ab')₂ fragment of an anti-sialyl Lewis a monoclonal antibody and clinical radioimmunoscintigraphy of recurrent rectal carcinoma patient

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An IgG1 mouse monoclonal antibody (MoAb) to sialyl-Lewis^a antigen that showed specific *in vitro* binding to human gastrointestinal tract tumors was obtained by the hybridoma technic. F(ab')₂ fragments of this MoAb were radioiodinated and injected into nude mice bearing human colon carcinoma xenografts. The MoAb fragments accumulated specifically in the colonic tumors at 1~2 days after injection and did not accumulate in an unrelated tumor (melanoma). The tumor/normal tissue ratio and tumor/blood ratio was 2~10 fold higher in mice with F(ab')₂ fragments as compared to mice injected with the intact MoAb, suggesting that the F(ab')₂ fragment could be used in radioimmunoscintigraphy. Based on this favorable result, clinical application was performed in a patient with pelvic recurrence of rectal carcinoma. The radiolabelled F(ab')₂ fragment produced a positive scan at 3days after administration.

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Key words : radioimmunolocalization, colon cancer, monoclonal antibody, sialyl-Lewis^a, F(ab')₂ fragment

Although the feasibility of using radiolabelled monoclonal antibodies (MoAb) for localizing tumors by external scintigraphy has been documented in a number of reports¹⁻⁴⁾, it is also common knowledge that significant background radioactivity remains for several days and makes radioimmunodetection difficult without computer-aided background subtraction. However, this type of imaging exposes the patient to additional radiation as well as the risk of mistakes at the time of data analysis. Antibodies of high specificity that are cleared more rapidly from the circulation are thus desirable, and elimination of the Fc portion of an antibody is reported to be of potential benefit in this respect⁵⁾. Better access to tumor cells and more rapid clearance from the circulation are expected for Fab or F(ab')₂ fragments when compared to intact MoAbs⁶⁾, encouraging further investigations to be performed.

In the present study, we compared the potential of an intact ¹²⁵I-labelled murine anti-sialyl Lewis^a MoAb (H-15) and its Fab and F(ab')₂ fragments for the imaging of a human colon cancer cell line (SW-403) xenografted into nude mice. The favorable results obtained led us to the clinical application of the F(ab')₂ fragment in a patient with recurrent rectal carcinoma.

Materials and methods

1. Immunolocalization of xenografted colon carcinoma

MoAb and human cancer cell line xenografts

An intact IgG1 kappa MoAb (H-15), which binds to sialylated Lewis^a antigen and is expressed by adenocarcinoma of the colon, stomach, pancreas, and breast, was prepared for the radioimmunolocalization of tumors as described previously⁹⁻¹¹⁾. Briefly, 4-6-week-old nude mice (nu/nu Balb/c) were injected subcutaneously in the left thigh with 1.0×10⁷ SW-403 human colon cancer cells and in the right thigh with SK-MEL-28 human melanoma cells. All mice received 0.1% (v/v) potassium iodide in the drinking water throughout the experiment from 72 hours before injection of the radiolabelled antibody in order to block the uptake of free radioiodine by the thyroid gland.

Purification of the MoAb and production of Fab and F(ab')₂ fragments

The MoAb was purified from mouse serum and from ascitic fluid by protein A Sepharose column chromatography¹²⁾. Fab fragments were prepared using 2% papain by a modification of the method of Porter¹³⁾. F(ab')₂ fragments were prepared using 2.5% pepsin by a modification of the method of Herlyn¹⁴⁾. The purity of the intact MoAb and its fragments was confirmed by 7.5% SDS-PAGE analysis using unreduced gels¹⁵⁾. Pepsin and papain were removed by dialysis and undigested IgG was removed by chromatography with

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Fig.1 Cellular binding of intact H-15 antibody and its F(ab')₂ and Fab fragments in mixed hemmagglutination assay.

Sephadex G-100 for Fab fragments or G-200 for F(ab')₂ fragments. Antibody and fragment concentrations were quantitated by the absorbance at 280nm.

Cellular binding of the intact MoAb and its fragments

Cellular binding of the intact H-15 MoAb and its Fab and F(ab')₂ fragments was tested by the mixed hemadsorption assay using rabbit anti-mouse immunoglobulin, as described previously¹⁰.

Biodistribution and radioimaging study in tumor-bearing mice

For the biodistribution and radioimaging study, a total of 36 nude mice bearing SW-403 tumors and SK-MEL-28 tumors weighing about 1g each were injected intraperitoneally with 0.1mCi (approximately 0.2mg) of ¹²⁵I-labelled H-15 or its fragments in phosphate-buffered saline containing 10% normal mouse serum (v/v).

For biodistribution studies, the mice received the ¹²⁵I-labelled intact MoAb (intact MoAb group) or its F(ab')₂ fragments (F(ab')₂ group). Two mice of each group were sacrificed and dissected at 2 and 6 hours after injection, and then daily until 7 days after injection. The samples of tumor, blood, viscera and muscle were weighed and assayed for radioactivity. Results were expressed as (a) : the ratio of the activity of the intact MoAb and its F(ab')₂ fragment in the colon carcinoma tissue to that in normal mouse tissue or melanoma [(cpm/g in tumor)/(cpm/g in blood, liver, muscle, or melanoma)] (b) : as the percentage of injected radioactivity per gram of tissue corrected for physical decay.

In the radioimaging study, images were recorded daily for up to 7 days after injection. Mice were anesthetized and images were obtained in the A-P and P-A views using a large field of view scintillation camera equipped with a medium-energy, parallel-hole collimator and interfaced to a computer. Each image

Fig.2 Tumor/muscle ratios after the injection of intact H-15 monoclonal antibody or its F(ab')₂ fragment labeled with ¹²⁵I.

was digitized and stored on disk for later analysis and display. No background subtraction or computer smoothing was performed.

2. Radioimmunoscintigraphy with the radiolabelled F(ab')₂ fragment in a patient with recurrent rectal cancer

The F(ab')₂ fragment of MoAb H-15 (0.5mg) was labelled with 2mCi of ¹³¹I sodium iodide by the chloramine T method and injected into a 77-year-old woman with pelvic recurrence of rectal carcinoma. Administration of the antibody, sequential assessment of urinary antibody excretion and radioimaging were performed as described previously¹¹.

Results

1. Radioimmunolocalization of xenografted colon carcinoma *in vitro* binding of the radiolabelled MoAb and its fragments

The cellular binding of the radioiodinated intact H-15 MoAb and its Fab and F(ab')₂ fragments was evaluated on the basis of the percent rosette formation in the MHA test (Fig.1). Radioiodinated intact H-15 retained its reactivity for the target SW-403 colon cancer cells at nearly a dilution of 10⁴, whereas the Fab fragments rapidly lost their reactivity (50% rosetting at a 10fold dilution). However, F(ab')₂ fragments retained considerable reactivity for the target cells at dilution of 5 × 10³. Iodinated intact H-15 and its F(ab')₂ fragment did not bind to SK-MEL-28 melanoma cells.

The results of this *in vitro* study suggested that utilization of the F(ab')₂ fragment was feasible for biodistribution and imaging studies.

Fig.3 Tumor/liver ratios after the injection of intact H-15 MoAb or its F(ab')₂ fragment labeled with ¹²⁵I.

Fig.4 Tumor/blood ratios after the injection of intact H-15 MoAb or its F(ab')₂ fragment labeled with ¹²⁵I.

In vivo distribution of ¹³¹I-labelled intact H-15 and its F(ab')₂ fragment

The colon cancer/muscle ratio (Fig.2), colon cancer/liver ratio (Fig.3), colon cancer/blood ratio (Fig.4) and colon cancer/melanoma ratio (Fig.5) were calculated at each time of sacrifice in both groups. As is evident from the results, colon cancer radioactivity was higher in both groups as compared to that in normal tissues or melanoma tissues following injection of the radioiodinated antibodies. The highest tumor/muscle ratio of 5 was observed on days 4~7 in the intact MoAb group and the maximum of 75 was seen on days 5~7 in the F(ab')₂ group (Fig.2).

The tumor/liver ratio reached its peak of 3 on days 5~7 for the intact MoAb group and it was as high as 25 on day 2 in the F(ab')₂ group (Fig.3). The tumor/blood ratio in the intact MoAb group was only 1.5~1.8 at its

Fig.5 Localization of ¹²⁵I-labeled F(ab')₂ fragments of MoAb H-15 24 hours (a) and 36 hours (b) after injection. No background subtraction or computer smoothing was used. The colon tumor is seen more clearly in the scan obtained at 36 hours (arrow). Each mouse weighed 25g.

Table 1 Percent antibody dose injected per gram of tissue (%ID) for the ¹²⁵I labelled intact MoAb H-15 and its F(ab')₂ fragments in nude mice bearing SW-403 colon cancer and SK-MEL-28 melanoma.

Tissue	intact MoAb	F(ab') ₂ fragment
Colon cancer	0.36	0.56
Melanoma	0.08	0.03
Blood	0.25	0.09
Liver	0.10	0.07
Colon	0.09	0.05
Muscle	0.06	0.02

highest on days 5~7, whereas in the F(ab')₂ group it reached a maximum of over 10 on days 5~7 (Fig.4).

The percent antibody dose injected per gram of tissue was investigated at 4 days after injection (Table 1). Both intact H-15 and its F(ab')₂ fragment demonstrated preferential localization in the human colon cancer cells as reflected by the high %ID. The %ID of all normal tissues tested ranged from 0.06 to 0.25 for the intact MoAb and from 0.02 to 0.09 for the F(ab')₂ fragment, whereas it was 0.36 for the intact MoAb and 0.56 for the F(ab')₂ fragment in the xenografted human colon cancer tissues. The %ID values in the human melanoma were 0.08 for the intact MoAb and 0.03 for its F(ab')₂ fragment and these were not significantly different when compared to normal tissue. The selectivity and specificity of the binding of ¹²⁵I-labeled F(ab')₂ fragments to human colon cancer cells suggested the possibility of performing radioimmunoimaging of such tumors.

Tumor localization by radioimaging with the ¹³¹I-labeled F(ab')₂ fragment of MoAb H-15

Radioimmunoimaging was performed in nude mice

Fig.6 Comparison of the urinary excretion of radiolabeled intact (closed circle) and F(ab')₂ fragments (open circle) of MoAb H-15 in a patient with recurrent colorectal cancer.

F(ab')₂ excretion was faster than that of the intact monoclonal antibody.

Fig.7 Localization of F(ab')₂ fragments of MoAb H-15 on day 3 in a patient with locally recurrent rectal cancer.

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.

grafted with human SW-403 colon cancer and injected with 100 μ Ci of ¹³¹I-labeled H-15 F(ab')₂ fragments. Tumor localization was possible without background subtraction. As early as 24 to 36 hours after F(ab')₂ administration, the tumors were visible on anteroposterior images (Fig.5), and they become increasingly clear throughout the study as the radioactivity in the rest of the body decreased. The maximal tumor contrast, as quantitated by analyzing digital computer images, was obtained between 36 hours (1.5 days) and 96 hours (4 days) after MoAb injection. Transient bladder and heart activity was observed during the early period of radioimaging (24 to 48 hours). None of the melanoma xenografts were visible at any time after the injection of H-15 F(ab')₂ fragments.

2. Radioimmunoscinigraphy with radiolabeled F(ab')₂ fragments in a patient with recurrent rectal cancer

Serial abdominal images were obtained from day 1 to day 5 after antibody infusion. Urine samples were collected daily after the injection of H-15 F(ab')₂ fragments and the percent excretion of radioactivity in urine was estimated (Fig.6). For reference, the mean and the 95% confidence interval for the urinary excretion of radiolabeled intact H-15¹¹⁾ is also displayed in the figure.

The radioimaging findings on day 3 are shown in Fig.7. The initial image obtained at 1 day after antibody injection corresponded to the blood pool and the tumor image became increasingly distinct with rapid clearance of the F(ab')₂ fragments from the blood. By day 3, the recurrent tumor was clearly distinguishable from the surrounding pelvic tissues and organs. The P-A image was clearer than the A-P image, implying the presence of radioactivity in the recurrent lesion and not in the bladder.

Discussion

In our previous study, the scintigrams obtained using intact antibody without background subtraction provided adequate tumor delineation¹¹⁾. However, there was a significant delay before clearance of the background radioactivity was sufficient to allow the tumor to be easily delineated.

The rationale for using the radiolabeled F(ab')₂ fragment instead of the intact MoAb was that loss of the Fc fragment should prevent binding to complement and to cells with Fc receptors such as macrophages⁸⁾. Therefore, we expected a lower nonspecific accumulation of radiolabeled antibodies in the normal tissues. In addition the small size of the F(ab')₂ fragment may facilitate its prompt uptake by target cells and more rapid clearance from the blood¹⁷⁻¹⁹⁾.

Our present radioimmunolocalization and dosimetry studies confirmed that the F(ab')₂ fragment was associated with low background radioactivity and faster clearance. Images obtained with this fragment delineated the tumor earlier and with less background radioactivity than when the intact MoAb was used. It might also be possible to use larger doses of F(ab')₂ than the intact MoAb in order to detect smaller tumor foci. Even with an increase in the dose, the radiation exposure to normal tissue could still be less than with the intact MoAb due to the faster clearance of the F(ab')₂ fragment.

However, the gradual but faster loss from the tumor of F(ab')₂ fragments as compared to the intact antibody might be a disadvantage if the long-term delivery of radiation or antibody-bound toxin was planned for

therapeutic purposes. It would be possible for this shorter tumor residence time of the F(ab')₂ fragment to be overridden by repeated administration if necessary.

Our results obtained in nude mice bearing transplanted human colon carcinoma and preliminary results obtained in a patient with rectal cancer showed that satisfactory radioimmunodetection of tumors was possible with and without simple background subtraction, and was much faster with the F(ab')₂ fragment than with the intact MoAb. In addition, the absolute tumor/tissue ratios were significantly higher for the F(ab')₂ than for the intact MoAb in the case of blood, liver, and muscle at several time points early after and these are certainly desirable qualities for scintigraphy²⁰⁻²³.

Based on these findings, we suggest that F(ab')₂ fragments may assume an increasingly important role for *in vivo* immunodiagnostic systems by decreasing the need for background subtraction, and enabling earlier imaging. They may possibly also be used, in immunotherapy as vehicles for toxin or radionuclide delivery.

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Rat model of bone metastasis obtained by mammary cancer transplantation

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骨転移に対する放射線の照射効果を実験的に調べるための動物モデルをつくることを目的として、近郊系のラットに発癌物質で発生させた乳癌細胞を骨髓腔に移植し、形態学的・X線学的検索を行い、骨転移ラットモデルの性質について検討した。

【対象と方法】乳癌(腺癌)は、近郊系雌性のFischer-344系のラットに7,12-dimethylbenz (a) anthracene (DMBA)を経口投与して発生させた。この乳癌を同系のラット皮下に移植して継代維持のできる移植性乳癌を得た。この移植性乳癌細胞を同系ラットの骨髓腔に注入して転移性骨腫瘍の動物モデルを作成した。

本研究では、乳癌細胞をラットの膝の部位から大腿骨の骨髓腔に移植した時の動物の生存率の測定、X線写真および組織病理学的検索を行った。また、乳癌細胞を移植した大腿骨に対するX線照射の影響も調べた。

【結果】本実験で使用したラット移植性乳癌細胞は、同系のラットの皮下あるいは骨髓腔に移植すると、移植したラットの全例に生着した。

100万個の乳癌細胞をラットの皮下および骨に移植したときの生存日数は、それぞれ 47 ± 24 日と 21 ± 11 日であった。乳癌細胞を移植された骨では、移植後約7日目頃からX線写真上に骨膜反応が認められた。

組織学的には移植後、内膜および骨膜表面の休止期の扁平な骨芽細胞層の近傍から多数の骨芽細胞と破骨細胞が発生した。腫瘍組織の増大に伴って、既存骨および新生骨の両部位における破骨細胞による骨溶解と腫瘍細胞による骨損傷が進行して、最終的に骨折や骨破壊が起こって動物は死に至る。乳癌細胞を骨に移植したラットでは、全例に乳癌の肺への転移が起こった。一方、同様に皮下に移植したラットでは、乳癌の肺への転移は認められなかった。乳癌細胞を移植した部位の骨をX線で照射するとラットの寿命は延長した。

Radioimmunolocalization of human colorectal carcinoma xenografts with $F(ab')_2$ fragments of anti-sialyl Lewis x monoclonal antibody (MoAb) and a case report of radioimmunoscintigraphy with the radiolabelled fragment on recurrent rectal carcinoma patient

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大腸癌培養細胞HT-29の細胞表面に発現しているシアリルLewis^x抗原に対するモノクローナル抗体(MoAb) H-15を精製し、ペプシン、ババインによりFab、 $F(ab')_2$ のfragmentを作成して、*in vitro*、*in vivo*の系においてdosimetry analysisを行い、腫瘍画像診断における有用性を検討した。

【対象と方法】*in vitro*の系では、whole MoAbとFab、 $F(ab')_2$ の抗原に対する親和性を大腸癌培養細胞株SW403をターゲットとして、mixed hemmagglutination test (MHA)

にて判定した。*in vivo*の系では、whole MoAbと $F(ab')_2$ の双方をクロラミンT法にて¹²⁵Iにより標識し、ヌードマウス腹腔内に投与し、その腫瘍：筋肉比、腫瘍：肝比、および腫瘍：血液比を抗体投与直後より7日目まで連日測定した。また、標識抗体投与後4日目には、移植大腸腫瘍、移植メラノーマ、血液、肝、正常大腸、および筋肉における% injected dose per gram (%ID)の測定も行った。

ヌードマウスにおける腫瘍画像診断は、¹²⁵I標識抗体(whole MoAbと $F(ab')_2$ fragment)投与後連日シンチレーションカメラを用いて、A-PおよびP-A画像の撮影を行った。

この結果をもとにして、直腸癌局所再発患者1例において¹²⁵I標識 $F(ab')_2$ 抗体を投与し、ガンマカメラを用いて抗体の局在を検討した。抗体投与による重篤な副作用は認められなかった。

【結果】ヨード標識をしたwhole MoAbとFab、 $F(ab')_2$ の大腸癌細胞株SW403に対するMHAテストではwhole MoAbが $\times 10^4$ 、Fabが $\times 10$ 、 $F(ab')_2$ が 5×10^3 の希釈まで反応を示した。この結果、H-15MoAbに関しては、放射性ヨード標識後はFab fragmentの抗原結合能がいちじるしく低下することが明らかになった。

*in vivo*の系においては、標識抗体投与後4~7日目において腫瘍：筋肉比がwhole MoAbで $\times 5$ 、 $F(ab')_2$ では $\times 75$ であり、腫瘍：肝比は、whole MoAbで $\times 3$ 、 $F(ab')_2$ で $\times 25$ 、腫瘍：血液比はwhole MoAbで $1.5 \sim 1.8$ であるのに対し、 $F(ab')_2$ では $\times 10$ と $F(ab')_2$ fragmentのより高い腫瘍特異的集積が明らかになった。また、%IDも $F(ab')_2$ は移植大腸腫瘍0.56 (whole MoAbでは0.36)、移植メラノーマ0.03 (0.08)、血液0.09 (0.25)、肝0.07 (0.10)、正常大腸0.05 (0.09)、筋肉0.02 (0.06)と移植大腸腫瘍部において高値を示していた。ヌードマウスにおける画像診断では、 $F(ab')_2$ 抗体投与後36時間で、移植大腸腫瘍部に抗体の集積がみられ、whole MoAbに比し、早期に腫瘍画像を得ることができた。また、直腸癌局所再発症例において $F(ab')_2$ を投与した1例では、投与後3日目において腫瘍画像が抽出され、臨床応用への有用性が示唆された。

【考察】wholeのMoAbに対し $F(ab')_2$ fragmentを腫瘍画像診断に用いることは、①早いclearance、②抗体の腫瘍へのaccess、③Fcによる非特異的集積の減少などの利点があるといわれている。

今回の検討により、*in vivo*、*in vitro*、また臨床の場合においても $F(ab')_2$ fragmentの高度な腫瘍特異的集積が証明され、今後の臨床応用への有用性が示されたものと考えられる。

Prognostic value of sialyl-Tn antigen in gastric carcinoma

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Kiyoshi Maeda et al.

近年、糖鎖抗原は腫瘍マーカーとして重要な位置を占めている。糖鎖性腫瘍マーカーの一つであるcarbohydrate antigen 19-9 (CA19-9)、sialyl Lewis^x-i (SLX)などは基幹糖鎖を抗原としたものであるが、これに対しシアリルTn