Basic Investigations

RADIOIMMUNODETECTION OF HUMAN CHORIOCARCINOMA XENOGRAFT IN NUDE MOUSE

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ABSTRACT

Objective: To study the efficiency of radioimmunodetection in locating the xenograft of human choriocarcinoma in nude mouse. Methods: Radioimmunodetection was performed using cocktail antibodies of ^{131}I-labeled mouse anti-human chorionic gonadotropin monoclonal antibodies to locate the xenograft of human choriocarcinoma in nude mouse. Radioactivity in different tissues was measured and the tumor/non-tumor ratio was calculated. Normal mouse IgG was used as control IgG. Results: The accumulation of radioactivity in the xenograft area could be recognized as early as 24 h after the injection of the radiolabelled antibodies. 72–96 h after the injection, the xenograft could be clearly shown. The minimal shown xenograft was 0.8 cm in diameter. The tumor/non-tumor ratio increased with the time and was obviously higher than that in control group. Conclusion: Radioimmunodetection can efficiently locate human choriocarcinoma xenograft in nude mouse.

Key words: Choriocarcinoma, Neoplasm transplantation, Disease models, Animal, Radioimmunodetection, Nude mouse

In order to collect data for further clinical study, we investigated the efficiency of radioimmunodetection (RID) in locating the xenograft of human choriocarcinoma in nude mouse using ^{131}I labeled mouse anti-human chorionic gonadotropin (hCG) monoclonal antibody (MAb).

Received May 26, 2000, accepted July 3, 2000

This work was supported by a grant from the National '863' Project of China (No. B3879028).

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MATERIALS AND METHODS

Preparation of Antibodies

Hybridoma ascites containing three strains of mouse anti-hCG MAbs was provided by the Monoclonal Antibody Lab of the Department of Endocrinology of Peking Union Medical College Hospital. These three strains of MAbs were purified by Hydroylapatide Chromatography in the Radioimmunoassay Lab of our department. Percentage Cross Reaction and Competitive Combination Test confirmed that these antibodies showed no cross reaction with human luteinizing hormone (hLH), human follicular stimulating hormone (hFSH), human thyroid stimulating hormone (hTSH) and the α subunit of hCG.[1] Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) showed one single band. Agar Gel Double Immunodiffusion suggested it was IgG1. Radioimmunoassay showed that the titer of the antibodies is 1:10000. The result of pyogen and toxin test was correspondent to the standard of the Chinese Pharmacopoeia. Cocktail antibodies were made by mixing these three strains of mouse anti-hCG MAb. The final concentration is 5 mg/ml. Normal IgG (NMIgG) (Provided by Beijing Bioproduct Institute) was used as control IgG.

Labeling of Antibodies

Under the alkaline condition (pH 7.4), antibodies were labeled with ^{131}I by Chloramine-T Method.[2] The reaction was stopped by Sodium Metabisulfite ($Na_2S_2O_5$). Then the product was put through Sephadex G-50

(Phamacia) chromatographic column, washed by Phosphate Buffer Solution (PBS). Immunoactivity of both labeled and unlabeled antibodies were measured by Enzyme Linked Immunosorbent Assay (ELISA).

Human Choriocarcinoma Xenograft in Nude Mouse

Fresh human choriocarcinoma 0.5 ml was injected subcutaneously in the groin area of the 4- week age female BALB/C nude mice (Provided by the Animal Center of Chinese Academy of Medical Science). The xenografts grew to 0.8–1.5 cm in diameter 10–14 days after the injection. The serum hCG of the tumor-bearing mouse is above 776 U/L. The xenograft tissue was examined pathologically, immunohistologicaly and electron-microscopically. And all the results confirmed that the xenograft was choriocarcinoma. The injected tissue of each mouse came from the tumor tissue of the same patient. The nude mouse was weighted 16–25 g. The xenograft was 15%–24% of the body weight.

Radioimmunodetection

Sixteen tumor bearing mice were used, 14 in trial group and 2 in control group respectively. 1% KI was added to the mice's drinking water 3 days before RID to block the thyroid gland of the mice. Every mouse in trial group was introperitoneally injected with ^{131}I labeled mouse anti-hCG MAb 10 g (2220–2590 kBg). Every mouse in the control group was introperitoneally injected with ^{131}I labeled NMIgG 10 g (2220–2590 kBg). RID was performed at 24th, 48th, 72ed, 96th and 120th h after the injection with a Sigma 420 γ camera (Sigma, USA) with a pinhole collimater. The RID condition: window width 20%, small field, counting to 20-30 K and saving to the floppy disk.

Tumor/Non-tumor Ratio

3, 3, 3, 2 and 3 mice in the trial group were killed at the 24th, 48th, 72ed, 96th and 120th h after the injection respectively. Mice in the control group were killed the 120 hours after the injection. Tissues of the xenograft, heart, the liver, the kidney, the lung, the stomach, the spleen and the muscle of the thigh of each mouse were dissected, washed by Normal Saline and carefully weighted. The radioactivity of these tissues was measured with a well γ detector. The radioactivity per gram of each tissue was calculated and the tumor/non-tumor radioactivity ratio (T/NT ratio) was calculated.

RESULTS

The Labeling of Antibodies

The labeling rate was 70%. The radioactivity was 185–296 kBg. The radiochemical purity is >90%. ELISA showed that above 80% of the immunoactivity of the antibodies were conserved after the radiolabeling. RIA showed the titer of the antibodies is >1:10000.

RID

The accumulation of radioactivity in the xenograft area could be recognized as early as 24 h after the injection of the radiolabeled antibodies in the trial group. The accumulation was getting more obvious with the time. The xenograft was clearly shown 72–96 h after the injection. The accumulation site was correspondent to the xenograft area. The minimal shown xenograft was 0.8 in diameter. The distribution of the radioactivity in the control group was event. There is no recognizable accumulation of radioactivity in the xenograft area in the control group at anytime after the injection of the radiolabeled NMIgG.

T/NT Ratio

As it was shown in Table 1, the T/NT ratios in the trial group was getting higher with the time after the injection. At the 72ed h after the injection, the T/NT ratios in the trial group were generally above 2 (kidney's 1.97). The T/NT ratios were different among different tissues at the given time. The T/NT ratios of the kidney, the liver, the lung and the spleen were relatively low. Aside from the T/NT ratio of blood, the T/NT ratio is the lowest. The T/NT ratios of the stomach, the heart and the muscle were relatively high. The T/NT ratio of the muscle is the highest. At the 120th h after the injection, the T/NT ratios in the trial group were all higher than the control group's. No statistic test was performed because of the relative small animal number. At 120th h after the injection, the T/NT ratios in the control group were generally >1 (tumor/blood 0.87). The highest was 2.54 of tumor/muscle

DISCUSSION

Mouse Anti-hCG Mab

Because the principle of RID is the antigen (Ag)-antibody (Ab) specific reaction, the characteristics of the used MAb will directly affect the result of RID. Generally speaking, using antibodies with high specificity, immunoactivity and affinity would improve the quality of RID. hCG is a protein hormone with double chains. Its α chain is identical to the α chain of hLH, hFSH and h TSH. So the ideal MAb's target antigen determining sites should be on the β chain.[1] In

RID, the MAb's target antigen is tumor associated antigen (TAA). There maybe several epitopes for each TAA. So, blending of antibodies targeting different epitopes of the same TAA will increase the Ag-Ab combination and raise the sensibility of RID.[3] The labeling method of MAb is another factor affecting the RID result. Chloramine-T is a simple, reliable labeling method with high labeling rate. But it may affect the immunoactivity of the Mab.[2] In our study, we used the cocktail antibodies of three strains of mouse anti-hCG MAb which showed no cross reaction with hLH, hFSH, hTSH and the α subunit of hCG. The labeling rate of Chloramine-T was 70%. ELISA showed above 80% of the immunoactivity of MAb was reserved after the radiolabeling. RIA shows the MAb titer is above 1:10000. The RID result is satisfying.

Table 1. T/NT ratios

Group	Time (h)	Number	Blood	Heart	Liver	Kidney	Lung	Stomach	Muscle	Spleen
Trial	24	3	0.44	2.39	0.74	1.02	1.22	1.45	2.67	0.91
	48	3	0.77	3.05	1.32	1.55	2.06	2.72	4.07	1.73
	72	3	1.08	3.81	2.05	1.97	2.61	3.33	5.26	2.15
	96	2	1.32	5.61	3.35	2.15	3.70	4.13	7.53	3.95
	120	3	1.68	6.88	4.55	2.52	4.72	5.09	8.48	5.78
Control	120	2	0.87	1.93	1.17	1.16	1.66	1.84	2.54	1.75

Human Choriocarcinoma Xenograft in Nude Mouse

Subcutaneous xenograft in nude mouse were used in most animal studies of RID. Even though these models can mimic the real tumor in human being to a certain degree, there are some problems. Because the nude mouse has no thymic gland, it demands a high quality living environment and the interactivity between RID and the immune system can't be observed. On the other hand, because the xenograft is just beneath the skin and the tumor/body ratio of the mouse is much higher than that of a cancer patient is, the RID quality in xenograft would surely be better than those in clinical practice.[4] In our study, tumor/body ratio of the nude mouse is 15%–20%. It's much higher than our patients' are.

The Effectiveness of RID

After the injection of the radiolabeled antibodies, the antibodies will be eliminated gradually and the radioactivity decreases. The radioactivity in the tumor sites, however, decreases much slower than those in the non-tumor area does, because the Ag-Ab reaction incurs the accumulation of the radiolabeled antibodies.[5] In our study, RID using ^{131}I labeled mouse anti-hCG MAb could effectively reveal the xenograft of human chorio-carcinoma in nude mouse. The accumulation of radioactivity in the xenograft area could be recognized as early as 24 h after the injection of the radiolabeled antibodies in the trial group. The accumulation was getting more obvious with the time and was clearly shown 72–96 hours after the injection. The distribution of the radioactivity in the control group was event. There is no recognizable accumulation of radioactivity in the xenograft area in the control group at anytime after the injection of the radiolabeled NMIgG. This result suggests that, compare to the non-specific immunoglobulin, ^{131}I labeled mouse anti-hCG MAb can specifically accumulated in the xenograft site. In our study, all the xenografts in the trial group were revealed and the minimal revealed one was 0.8 cm in diameter. As we mentioned above, the efficiency of RID in animal models will be more satisfactory than what is shown in clinical use.

The Distribution of Radiolabeled Antibodies

The T/NT ratio, to some extent, showed the strength of the accumulation of the antibodies in the tumor sites. In our study, the T/NT ratios in the trial group were getting higher with the time after the injection. At the 72ed h after the injection, the T/NT ratios of the major organs in the trial group were generally above 2 (kidney 1.97). At the 120th h after the injection, the T/NT ratios in the trial group were all higher than the control group's. This fact suggests that ^{131}I labeled mouse anti-hCG MAb can specifically accumulate in the tumor sites. Because the T/NT ratios will be getting higher with the time after the injection of the radiolabeled antibodies, the RID quality will be better at a relatively later time after the injection of the radiolabeled antibodies. However, it is not necessary to delay the RID too much. In our study, satisfactory images were acquired 72–96 h after the injection of the radiolabeled antibodies.

Data in Table 1 suggest that the T/NT ratios be relative to the blood flow of a given organ. Aside from

the blood's, the T/NT ratio of the kidney was the lowest. This phenomenon is possibly caused by the relatively rich blood flow of the kidney and its urination function. The T/NT ratios in other organs with relatively rich blood flow, such as the liver, the lung and the spleen, were also relatively low. So it is difficult to use RID to reveal the tumors (or the metastasizes) in these organs. The T/NT ratios of the organs with less blood flow, such as the stomach and the heart (blood was wash out), were relatively high. The T/NT ratio of the muscle, whose blood flow is relatively not rich, was the highest. This means the quality of RID for organs with relatively less blood flow will be more satisfactory than those for organs with relatively rich blood flow. (RID for heart is not satisfactory because the living heart is full of blood.)

It is worth to notice that T/NT ratios of some organs in the control group could also reach relatively high degree at the 120th h after the injection of radiolabeled NMIgG. This phenomenon suggests that non-specific immunoglobulin also can accumulated to some degree in the tumor sites. It is generally believed that this is caused by high permeability of the blood vessel and obstruction of the lymph duct in the tumor area. Some authors believe that this kind of non-specific accumulation of the immunoglobulin is another promoting factor for the accumulation of the radiolabeled antibodies in the tumor sites in RID.[6]

Thyroid gland can accumulate the ^{131}I that was used in our study. Because the thyroid gland of the nude mouse is too tiny to be properly dissected and is easily confused with surrounding tissue, it is difficult to correctly measure the radioactivity of this organ. In our study, we didn't measure it.

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