Anti-human AFP variant monoclonal antibody in radioimmunodetection of primary hepatocellular carcinoma

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INTRODUCTION
Radioimmunodetection (RAID) and radioimmunotherapy using antibodies against tumor-associated antigens (TAA) are hot topics of tumor targeting research. Since the 1980s, several articles describing such anti-tumor monoclonal antibodies (McAb) have been published. Successful RAID and radioimmunotherapy with the anti-human AFP variant McAb (AFP-R-LCA McAb) against human hepatocellular carcinoma (HCC) were carried out in our laboratory using nude mice xenografts [1]. Based on our findings from those animal experiments, we next used the AFP-R-LCA McAb labeled by 131I to immunodetect 17 human cases of HCC and 4 cases of hepatitis B-related liver cirrhosis.

MATERIALS AND METHODS
Cases and groups
HCC was diagnosed according to clinical findings from CT or MRI, B ultrasonography or AFP concentration in serum, and pathological examinations (after operation). Liver cirrhosis was diagnosed according to clinical findings from B ultrasonography, CT or MRI, liver function test, etc. The pre-RAID clinical characteristics are shown in Table 1. All patients included in this study had been admitted to our hospital between March 1, 1995 and September 1, 1996.

Preparation of 131I-AFP-R-LCA McAb
The purification procedure for AFP-R-LCA McAb from ascites fluid was adopted from an earlier study [2]. Briefly, the monoclonal immunoglobulin IgG-containing ascites fluid was precipitated by saturated ammonium sulfate and the purified IgG was separated with a DEAE-Sephadex column. The monoclonal IgG was radioiodinated to a high specific activity with 131I using the chloramine-T method. The product of 131I-AFP-R-LCA McAb was isolated by a Sephadex-G column and then passed through a 0.22 μm filter to remove any bacterial contaminants. After cultivation and Limulus testing to ascertain the absence of bacteria and pyrogens, the product was prepared for clinical use. The labeling rates were 51% to 60%, and the specific activities were 0.11 to 0.33 GBq/mg.

Liver function, thyroid gland function, and prothrombin time (Pt)
Periphery vein blood was collected at 1-3 d before and 2 wk after the RAID.

METHODS of RAID
Seven days before and 1 d before the RAID, patients were administered a compound iodine solution to block the thyroid gland. Thirty min before the RAID, patients were injected with 25 mg of phenergan intramuscularly. The radioisotope-labeled monoclonal antibodies, 131I-AFP-R-LCA McAb, were injected intravenously through peripheral veins (3.7-7.4 × 107 Bq/300 μg). The upper abdomen was scanned using emission CT (ECT ILC 3700; Siemens, Germany) and photograms were taken (Omega 500 γ camera) at 24 h, 48 h, 72 h, 120 h and 144 h later.
The heart and spleen have a large blood supply, and the blood and spleen belong to the reticuloendothelial system and have a strong non-specific affinity to $^{131}$I-AFP-R-LCA McAb. Therefore, the heart and the spleen showed densely under ECT imaging.

Most of the AFP-positive HCC cases in the current study were not imaged because of large tumor size (>10 cm) with poor blood supply or related necrosis and higher serum AFP concentration (200000 µg/L); these features can thwart $^{131}$I-AFP-R-LCA McAb competitively to produce a large amount of immune complexes. The immune complex, itself, can hinder $^{131}$I-AFP-R-LCA McAb from getting into the tumor area. Thus, a clear image depends not only on the TAA concentration in serum but also on the tumor's blood supply.

Goldenberg et al reported that the serum concentration of AFP may have no influence on RAID, possibly because AFP on the surface of HCC cells is different from AFP circulating in the blood. This conclusion, however, is not consistent with ours.

Successful localization of a radioisotope-labeled monoclonal antibody to a tumor in vivo depends not only on the affinity of the antibody for the target cells but also on the speed with which the immunoconjugate passes through the physiological barriers (i.e. resistance of the blood vessel wall and phagocytosis by histiocytes) and the speed of its entering into the tumor tissues, as well as the antigen concentration in serum. It is a generally accepted practice that tumor images be obtained at 72 h after intravenous infusion of the antibody ligand, but that more distinct images are found within 120 h. Our experimental result agreed with this.

In conclusion, AFP-R-LCA-McAb has a strong and special affinity to AFP-positive HCC cells. The detection of AFP-R-LCA-McAb in tumor tissues of HCC suggests its potential as a carrier for RAID and radioimmunotherapy.

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