Monoclonal antibody against antigen A7 coupled to ferromagnetic lignosite particles

A7-FML

Arvind Chopra, PhD[1]

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Chemical name:	Monoclonal antibody against antigen A7 coupled to ferromagnetic lignosite particles	
Abbreviated name:	A7-FML	
Synonym:		
Agent Category:	Monoclonal antibody	
Target:	Antigen A7	
Target Category:	Antigen	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of Signal/Contrast:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structural information is available for this molecule.

Background

[PubMed]

According to recent publications, colorectal cancer (CRC) is the third most common cancer detected in men and women in the United States (1). The incidence of this ailment is believed to depend on an individual's genetic characteristics on the basis of sex, race, and location of the cancerous tumor(s) in the colon (2). Although several options are available for the treatment of this cancer, surgical resection is the most common treatment for this disease, and laparoscopic surgery is being actively debated as a

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

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treatment option (3, 4). The recurrence rate for CRC depends on the disease stage and can be >20% (4). Recurrence of the cancer generally indicates a poor outcome for the patient because recurrence of this cancer is usually detected at an advanced stage (5). Although the use of imaging techniques (such as ultrasonography, computed tomography, colonoscopy and magnetic resonance imaging (MRI)) is an option for the diagnosis of CRC, these techniques are not often used to make clinical decisions due to insufficient accuracy and often a combination of the different methods is used to arrive to a diagnostic conclusion (6, 7).

A noninvasive technique such as radioimmunoscintigraphy is now widely used for the diagnosis of cancers, and several monoclonal antibodies (MAbs) or fragments derived from these immunoglobulins are now in development or commercially available for this purpose (8). A clear advantage of the use of MAbs for imaging over other techniques is the targeted specificity of these agents and, in addition, the variety of nuclides that can be used to label these proteins for cancer/tumor therapy or imaging. Several MAbs have been approved by the United States Food and Drug administration for therapeutic and imaging applications in the clinic (9, 10). In an effort to develop a MAb that can be used for the diagnosis of CRC, Kotanagi et al. generated a MAb, designated A7, that reacted with 10 CRC tissues and 2 normal colon mucosa isolated from 19 CRC patients (11). The MAb was shown to bind to a 45-kD antigen characterized as a glycoprotein on the surface of human SW116 and WiDr colon cancer cell lines (further characterization of this antigen was not reported) (12). The MAb had weak or no reactivity to gastric, pancreatic, or lung cancer cell lines. The antigen binding MAb A7 was also detected in the colon surgical specimens of CRC patients, indicating that the MAb was specific for an antigen on CRC cells. Kitamura et al. showed that the antigen binding to MAb A7 was not similar to the carcinoembryonic antigen detected earlier in CRC cells (12). With a renewed interest to develop this MAb for the diagnosis of CRC, MAb A7 was coupled to supraparamagnetic iron oxide particles and used successfully as a contrast agent for the detection of rectal carcinoma with MRI (13). Otsuji et al. recently showed that MAb A7 could also be coupled to ferromagnetic lignosite (FML) particles to obtain A7-FML; MAb A7 can also be used to differentiate CRC tissue from normal or other tissues with the use of MRI in nude mice bearing human colorectal carcinoma WiDr cell xenografts (5). In the same study, the biodistribution of A7-FML was investigated in the xenograft mice using Mab A7-FML labeled with radioactive iodine (^{125}I).

Synthesis

[PubMed]

The hybridoma to produce MAb A7 was generated as described by Kotanaga et al. (11). To produce the MAb, ascites fluid was collected from BALB/c mice injected with hybridomas producing MAb A7, and the MAb was purified as detailed by Kitamura et al. (12). The isolated MAb was then dialyzed against phosphate-buffered saline (PBS) and stored at -70°C until required.

Radiolabeling of MAb A7 with ^{125}I was performed according to the chloramine T method as described elsewhere (14). The specific activity of the labeled MAb was reported to be 37 kBq/6.6 pmol (1.0 μCi/6.6 pmol). To couple the labeled MAb to FML, the radiolabeled MAb was added to FML in PBS and sonicated on ice (duration of sonication was not reported) (5). The sonicated particles were subsequently exposed to bovine serum albumin to mask any additional binding sites on the FML particles. The mixture was then centrifuged at 400,000 *g* for 15 min to obtain a pellet and remove any unbound labeled MAb. The pellet was then thoroughly washed with Hepes buffer (pH 7.4). The binding efficiency of FML for the labeled MAb was reported to be ~40%. For use as a control, normal mouse IgG was bound to FML using the same procedure outlined above. The storage conditions and stability of the FML-bound MAbs was not reported (5).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The binding of ^{125}I-labeled MAb A7 was investigated with a panel of human cancer cell lines generated from colon, gastric, pancreatic, and lung cancer tissue as described by Kitamura et al. (12). Only the colon cancer cell lines SW1116 and WiDr were reported to bind the labeled MAb.

In another study, Otsuji et al. studied the binding of ^{125}I-labeled A7-FML with WiDr cells in the presence of increasing concentrations of excess unlabeled A7-FML, and ^{125}I-labeled MAb A7 in excess of unlabeled MAb A7 for 60 min at 37°C (5). In control experiments, normal mouse ^{125}I-labeled IgG-FML was used instead of A7-FML or free A7. Both A7-FML and the MAb A7 labeled with ^{125}I were reported to have a similar binding activity with the WiDr cells. In comparison, the ^{125}I-labeled IgG showed negligible binding to the cells under these conditions. No binding affinities for A7-FML or the MAb A7 were reported by the investigators.

Animal Studies

Rodents

[PubMed]

The biodistribution of ^{125}I-labeled A7-FML was investigated in nude mice bearing WiDr cell xenograft tumors (5). The mice (n = 48) were divided into two groups with an equal number of animals and injected intravenously with either ^{125}I-labeled A7-FML or ^{125}I-labeled IgG-FML. The mice (n = 4/time point) from each group were euthanized and dissected at 2, 6, 12, 24, 48, and 72 h after the injections, and the various organs (heart, lung, spleen, liver, kidneys, pancreas, and colon), tumors, and blood were harvested to determine the accumulated radioactivity. The ratio of accumulated radioactivity in the tumor and normal tissue or blood, respectively, was calculated. Both ^{125}I-labeled A7-FML and ^{125}I-labeled IgG-FML showed a similar blood clearance curve from 2 to 72 h after administration. An increased accumulation of ^{125}I-labeled A7-FML was reported in the

tumor up to 24 h after injection, and a slow decrease in the label was observed subsequently up to 72 h. In comparison, a gradual decrease in the accumulation of ^{125}I-labeled IgG-FML was observed during the duration of the experiment (from 2 to 72 h) after injection. The tumor/blood ratio for ^{125}I-labeled A7-FML increased gradually to 2.59 ± 0.21 at 72 h. Although a similar trend with the tumor/blood ratio was observed with ^{125}I-labeled IgG-FML, the ratio at 72 h for the normal mouse labeled IgG compared with the labeled A7-FML was only 0.39 ± 0.14.

Mice were injected with either A7-FML (n=3 animals) or normal mouse IgG-FML (n=3 animals) and imaged with a MRI scanner using a T2*-weighted gradient echo sequence (5). The parameters used were: time/echo = 323/8.9 ms, flip angle 60°, bandwidth 31.2 Hz, matrix 512 × 512, field view 24 cm, two acquisitions in 11 slices (each with a thickness of 5 mm), and a scan time of 5.5 min. Reduced signal intensity was reported on the periphery of tumors only in mice injected with A7-FML. No such change in signal was observed with tumors of mice treated with normal mouse IgG-FML. This study demonstrated that A7-FML could be a suitable CRC detection and diagnostic agent using MRI.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

Supplemental Information

[Disclaimers]

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