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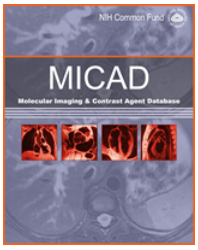

^{124}I-Labeled anti-prostate stem cell antigen affinity-matured A11 minibody

^{124}I-A11

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Chemical name:	^{124}I-Labeled anti-prostate stem cell antigen affinity-matured A11 minibody	
Abbreviated name:	^{124}I-A11	
Synonym:	^{124}I-Labeled A11 minibody	
Agent Category:	Antibodies (minibodies)	
Target:	Prostate stem cell antigen (PSCA)	
Target Category:	Antigens	
Method of detection:	Positron emission tomography (PET)	
Source of signal / contrast:	^{124}I	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

The use of intact antibodies as molecular imaging agents has some disadvantages, such as large size, poor tumor penetration, and immunogenicity (1-4). To minimize these problems, various formats of antibody fragments have been designed with the variable regions of the light chain (V_L) and the heavy chain (V_H) (1, 5, 6). Monovalent single-chain Fv (scFv) constructs (molecular weight, 25–30 kDa) represent the smallest antibody fragments, which are generated with V_L and V_H connected by a flexible linker. Although monovalent scFv fragments exhibit efficient tumor penetration, they are cleared rapidly from blood and can demonstrate poor antigen binding. For example, the β half-life of monovalent scFv in plasma is only 0.5–2.0 h (in contrast to 48–72 h for intact antibodies) (1). Bivalent antibodies such as diabodies (molecular weight, 55–60 kDa) and minibodies (molecular weight, ~80 kDa) are ideal for tumor targeting because of their compact sizes and better antigen binding compared to monovalent scFv fragments (1, 7). Diabodies are dimeric molecules consisting of two scFv fragments connected with a short linker. Minibodies are formed by the fusion of scFv fragments with the

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immunoglobulin G1 (IgG1) C_H3 domain. However, decreased antigen binding remains an issue for most antibody fragments compared with intact antibodies (1, 6, 8).

In previous studies, Olafsen et al. produced a humanized anti–prostate stem cell antigen (PSCA) intact antibody (hu1G8) and evaluated the antibody as an imaging agent (3). ^{124}I-Labeled hu1G8 has been shown to specifically accumulate in LAPC-9 prostate tumor xenografts; however, it takes 1 week to reach the maximal tumor/background signal. To improve the antibody pharmacokinetics for imaging use, Leyton et al. engineered several antibody fragments on the basis of the hu1G8 sequence (7). The hu1G8 minibody (scFv-C_H3 dimer; molecular weight, 80 kDa) is one of the fragments, and shows rapid blood clearance kinetics (MICAD chapter on ^{124}I-anti-PSCA 2B3 minibody). However, the apparent affinity of the hu1G8 minibody is only 5 nmol, representing a nine-fold loss compared with the apparent affinity of the intact hu1G8 antibody (46 nmol) (7). To increase the hu1G8 minibody affinity, Lepin et al. generated three affinity-matured minibody variants (A11, A2, and C5) with molecular evolution associated with yeast display, a powerful strategy for antibody affinity maturation (1). This strategy enables selection of scFv fragments with higher affinity from a yeast library generated with error-prone polymerase chain reaction (PCR). Lepin et al. demonstrated that the A11 variant has a higher affinity to PSCA than the parental hu1G8 minibody and exhibits more favorable pharmacokinetics for tumor imaging than other variants (1).

Related Resource Links:

- MICAD chapters on PSCA-targeted agents
- MICAD chapters on antibody fragments
- Gene information on human PSCA
- PSCA articles in OMIM

Synthesis

[PubMed]

Synthesis of the anti-PSCA mouse and human 1G8 intact antibodies and hu1G8 minibody has been reported previously and can be found in the MICAD chapter on ^{124}I-anti-PSCA 2B3 minibody as well (3, 6, 7, 9). Lepin et al. described the synthesis of the affinity-matured A11 minibody on the basis of the parental hu1G8 minibody (1). Briefly, the V_L and V_H coding sequences of the parental hu1G8 minibody were extracted and fused with an 18-residue GlySer-rich linker to form scFv in the V_L–V_H orientation in the pYD2 expression vector. Random mutations were introduced into the scFv by error-prone PCR to construct a library of scFv variants on a yeast display. The library was subjected to four rounds of equilibrium-based selection. A11 was one of the three selected clones, possessing six mutations in the complementarity determining regions (CDRs): CDR 3 of V_L and CDR 2 of V_H. The introduced mutations included a tyrosine, which is commonly used for iodination. To generate the A11 minibody, the A11 scFv variant sequences were fused to a signal peptide upstream and to the human IgG1 hinge and C_H3 region downstream. Two million mouse myeloma cells were transfected, and clones were screened for expression. Soluble minibodies were purified from the cell culture supernatants with protein L chromatography. The best yield was 30 mg/l when expanded to terminal cultures into 2% fetal bovine serum (FBS). The purified minibody was radioiodinated with the Iodogen method. The labeling efficiency values were 89.9% and 86.6% for the parental hu1G8 and A11 minibodies, respectively. The molecular weight of each minibody was ~80 kDa, corresponding to a covalently linked minibody. The radiochemical purity and specific activity were not described.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The stability of A11 minibody was evaluated after 5 days incubation either at 4°C in phosphate-buffered saline (PBS) or at 37°C in 5% FBS. The activity showed a decrease of 12% and 13% in PBS and FBS, respectively, compared with frozen sample (1).

The affinity of the A11 minibody was tested against PSCA-mγ2a recombinant protein with non-labeled intact monoclonal antibody 1G8 as the competitor. The A11 minibody exhibited a higher affinity than the parental minibody (16 nmol *versus* 50 nmol). However, both minibodies showed lower affinity than the mouse monoclonal antibody 1G8.

The affinity of the A11 minibody was further tested with PSCA-transfected SKW6.4-PSCA (B-cell lymphoma) cells after incubation of the radioiodinated minibodies with an excess amount of cells for 1 h at room temperature. The immunoreactive fraction for the A11 minibody (66%) was significantly greater ($P < 0.05$) than that for the parental minibody (45%) (1).

Animal Studies

Rodents

[PubMed]

Lepin et al. evaluated the A11 minibody for its biodistribution and its effectiveness in positron emission tomography (PET) imaging in mice bearing PSCA-expressing LAPC-9 human prostate tumors and Capan-1 human pancreatic tumors (1). The ^{124}I-labeled minibodies were administered through the tail vein of mice. Thyroid uptake of radioiodine was blocked with Lugol administered with the minibodies. Stomach uptake of the radiolabeled minibodies was blocked with potassium perchlorate administered *via* gastric lavage 30 min before minibody injection.

In the LAPC-9 tumor model ($n = 3$ mice/group), the A11 minibody demonstrated better biodistribution and imaging results overall than the parental hu1G8 minibody. The kidney and liver uptakes were 1.3 ± 0.02% injected dose per gram tissue (ID/g) and 0.8 ± 0.04% ID/g, respectively, for ^{124}I-A11, and 1.9 ± 0.41% and 1.3 ± 0.41% ID/g, respectively, for the parental minibody. Similar tumor uptake values were observed for both the A11 minibody (3.8 ± 0.27% ID/g) and the parental minibody (3.9 ± 0.26% ID/g) at 21 h after injection; however, a difference between them was observed for the tumor/blood ratio (1.1 *versus* 0.8) and for the tumor/background ratio (5.7 *versus* 2.4). The tumor imaging contrast was more than two-fold better with the A11 minibody than with the parental minibody.

Similar findings were obtained in the Capan-1 tumor model ($n = 5$ mice/group). The A11 minibody exhibited a slightly better tumor/blood ratio (0.96) than the parental minibody (0.8). A higher tumor/background ratio was reached with the A11 minibody (4.2) than with the parental minibody (2.2) ($P < 0.001$).

The investigators concluded that the ^{124}I-A11 minibody is better than the parental minibody for PET imaging of PSCA-expressing tumors (1).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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