^{99m}Tc-Mercaptoacetyl-Glu-Glu-aptamer specific for tenascin-C

^{99m}Tc-TTA1

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Chemical name:	^{99m}Tc-Mercaptoacetyl-Glu-Glu-aptamer specific for tenascin-C	
Abbreviated name:	^{99m}Tc-TTA1	
Synonym:		
Agent category:	Nucleic acid	
Target:	Tenascin-C	
Target category:	Nucleic acid binding protein	
Method of detection:	Single-photon emission computed tomography (SPECT)	
Source of signal/contrast:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Tenascin-C is a large, adhesive, extracellular matrix (ECM) glycoprotein (>10^3 kDa) (1) with numerous alterative names such as myotendinous antigen, glioma mesenchymal ECM protein (GMEM), cytotactin, J1-200/220, hexabrachion, and neuronectin (2). Tenascin-C contains six disulphide-linked subunits spread as six arms from a central core (3). Each subunit possesses multiple-function domains, including a cysteine-rich N-terminal domain associated with self-oligomerization, followed by epidermal growth factor (EGF)-like repeats, fibronectin (FN) type III-like repeats, and a fibrinogen-like

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globular domain at the C-terminus. Consequently, tenascin-C can bind to different cell-surface receptors such as integrins and ECM components (FN). Tenascin-C is found to express specifically in damaged tissues, vascular diseases, and a majority of malignant solid tumors (4).

Aptamers (from the Latin *aptus*, to fit, and the Greek *meros*, part or region) are single-stranded or double-stranded oligonucleotides (DNA or RNA) that are modified to bind a variety of targets with high binding affinity and specificity (5). Aptamers typically consist of 20–50 nucleotides (8–15 kDa) with dissociation constants in the range of 10 pM to 10 nM (6). Unlike linear oligonucleotides, which contain genetic information or antisense oligonucleotides that interrupt the transcription of genetic information, aptamers are globular molecules with a shape similar to tRNA and bind to target proteins specifically (7). The modification of the oligonucleotides is carried out through the systematic evolution of ligands by exponential enrichment (SELEX) method, in which specific aptamers are generated against desired small proteins, cells, and tissues (8). In general, aptamers are capable of distinguishing between closely related members of a protein family and between different functional or conformational states of the same protein (6). Aptamers have been used in various clinical trials as alternate therapeutics in cancers (6). For *in vivo* applications, aptamers can be modified chemically against degradation caused by exonucleases and endonucleases (9).

TTA1 is a 39mer oligonucleotide (13.4 kDa) that binds specifically to human tenascin-C with high affinity (dissociation constant, 5×10^{-9} M) (9). To enhance its resistance to blood and tissue nucleases, the structure of TTA1 is modified in several locations (9). All pyrimidine ribonucleotides are replaced with 2'-deoxy-2'-fluoronucleolytic nucleotides, 14 of the 19 purine ribonucleotides are replaced with 2'-deoxy-2'-OMe nucleotides, the 3' end is blocked with a 3'-3'-thymidine cap for exonuclease protection, a $(CH_2CH_2O)_6$ spacer is connected between oligonucleotide chains, and a 5' hexyl-aminolinker is added for bioconjugation of imaging probes. Connecting this aminolinker with radionuclide chelators, technetium-labeled mercaptoacetyl-Glu-Glu (^{99m}Tc-MAG_2), yields the molecular imaging agent ^{99m}Tc-mercaptoacetyl-Glu-Glu-aptamer specific for tenascin-C (^{99m}Tc-TTA1) (8). ^{99m}Tc is a common radioactive label used in single-photon emission computed tomography (SPECT) imaging, and it has a 0.90 gamma branch factor, a 6-h half-life at 140 keV, and a low isotope cost ($0.21/mCi) (10). ^{99m}Tc-TTA1 appears to be a promising candidate for imaging of tumors expressing tenascin-C (5).

Synthesis

[PubMed]

The synthesis of ^{99m}Tc-TTA1 was conducted in three steps (8). First, TTA1 was prepared according to standard protocols on an oligonucleotide synthesizer with the use of 2'-fluoropyrimidine phosphoramidite monomers, 2'-OMe purine, and $(CH_2CH_2O)_6$ monomers as building blocks (9). Then, TTA1 was reacted with mercaptoacetyl diglycine (MAG_2) succinimidyl ester at a molar ratio of 1:5 at pH 9.3, followed by purification with

reverse-phase liquid chromatography (8). Finally, the produced TTA1-MAG_2 was reacted with pertechnetate (185 MBq ^{99m}Tc in the presence of $SnCl_2$ at pH 8.5 to produce a radionuclide-labeled aptamer, ^{99m}Tc-TTA1 (8). The product was purified with spin-dialysis through a 10-kDa cutoff membrane filter. This labeling protocol incorporated 30%-50% of the added ^{99m}Tc, with a specific activity of 74-111 MBq/nmol of oligonucleotide. The labeling did not appear to alter the affinity of TTA1 binding to tenascin-C.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The uptake and localization of TTA1 was examined in tumors with TTA1 labeled with fluorescence dye (TTA1-Red) (5). Nude mice bearing human glioblastoma (U251) tumors (200–350 mg) were injected with TTA1-Red at a dose of 5 nmol (3.25 mg/kg). Mice were euthanized at several time points to extract the tumors for fluorescence microscopic imaging and immunohistochemical analysis. The antibody staining demonstrated an abundant presence of tenascin-C in the tumor stroma, including perivascular distribution. Ten minutes after injection of TTA1-Red, an intense fluorescence was found in the perivascular space; 3 h later, the fluorescence signal was diffused throughout the tumor stoma in a distribution pattern similar to that of tenascin-C in the ECM. Approximately 60% of aptamer in the tumor was found intact, suggesting that the fluorescence signal derived from the labeled aptamers.

Animal Studies

Rodents

[PubMed]

The biodistribution of ^{99m}Tc-TTA1 was measured with a gamma counter in tumor-bearing mice (5). Nude mice with U251 tumors (200–350 mg) implanted on the flank were used in the study. A mixture of 0.5 nmol ^{99m}Tc-TTA1 (37–55.5 MBq) with 4.5 nmol TTA1 was injected into the tail vein. The blood and tissues were collected at several time points for analysis of gamma radioactivity. ^{99m}Tc-TTA1 was found to clear from the blood rapidly: it decreased to 18% injected dose/gram of tissue (% ID/g) at 2 min after injection and decreased to 0.1% ID/g at 60 min. Tumor uptake was 5.9 ± 0.6% ID/g at 2 min, 2.7% ID/g at 60 min, and 1.2% ID/g at 17 h. The tumor/blood ratio was ~50 at 3 h and ~180 at 6 h. At 10 min after injection, ^{99m}Tc-TTA1 accumulation was found to be 19% ID/g in the kidney, 15% ID/g in the small intestine, and 10% ID/g in the liver. Three hours later, the level of ^{99m}Tc-TTA1 was 0.2% ID/g in the kidney, 1–1.55% ID/g in the small intestine, and 35–40% ID/g in the large intestine. These results suggested that ^{99m}Tc-TTA1 was cleared from body through the renal pathway (~50%) and the hepatobiliary pathway (~50%). No detectable radioactivity was found in blood, lung, or muscle at 3 h. More than 95% of ^{99m}Tc was cleared from body in 24 h. The uptake of

^{99m}Tc-TTA1 was examined in a variety of solid tumors (5) such as human tumor xenografts including colon cancer (SW620), breast cancer (MDA-MB-468, MDA-MB-435), rhabdomyosarcoma (A673), and squamous cell carcinoma. The radioactivity was found to be 0.1–1.9% ID/g at 3 h, depending on the tumors. The uptake of ^{99m}Tc-TTA1 was further examined *in vivo* with a gamma camera. Scintigraphic images were collected from mice bearing U251 tumors at the same time point at which images were collected for the biodistribution measurement. The results were consistent with the biodistribution data. No blocking study was reported.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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