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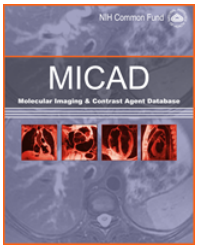

^{99m}Tc-Hydrazinonicotinic acid-single-chain Cys-tagged vascular endothelial growth factor-121

^{99m}Tc-HYNIC-scVEGF$_{121}$

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Chemical name:	^{99m}Tc-Hydrazinonicotinic acid-single-chain Cys-tagged vascular endothelial growth factor-121	
Abbreviated name:	^{99m}Tc-HYNIC-scVEGF$_{121}$	
Synonym:		
Agent Category:	Polypeptide	
Target:	VEGF receptors	
Target Category:	Receptor-ligand binding	
Method of detection:	Single-photon emission computed tomography (SPECT)	
Source of signal:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about VEGF.

Background

[PubMed]

Vascular endothelial growth factor (VEGF) consists of at least six isoforms with various numbers of amino acids (121, 145, 165, 183, 189, and 206 amino acids) produced through alternative splicing (1). VEGF$_{121}$, VEGF$_{165}$, and VEGF$_{189}$ are the forms secreted by most cell types and are active as homodimers linked by disulfide bonds. VEGF$_{121}$ does not bind to heparin like the other VEGF species (2). VEGF is a potent angiogenic factor that induces proliferation, sprouting, migration, and tube formation of endothelial cells. There are three high-affinity tyrosine kinase VEGF receptors on endothelial cells (VEGFR-1, Flt-1; VEGFR-2, KDR/Flt-1; and VEGFR-3, Flt-4). Several types of non-endothelial cells such as hematopoietic stem cells, melanoma cells, monocytes, osteoblasts, and pancreatic β cells also express VEGF receptors (1).

VEGF receptors were found to be overexpressed in various tumor cells and tumor-associated endothelial cells (3). Inhibition of VEGF receptor function has been shown to inhibit pathological angiogenesis as well as tumor growth and metastasis (4, 5). Radiolabeled VEGF has been developed as a tracer for imaging solid tumors and

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angiogenesis in humans (6-8). Cys-tag, a fusion tag comprising 15 amino acids, was developed for site-specific conjugation *via* the free sulfhydryl group of Cys. Backer et al. (9) prepared a Cys-tagged vector of $VEGF_{121}$ by cloning two single-chain fragments (amino acid sequence 3–112) of $VEGF_{121}$ joining head-to-tail to express as scVEGF, which can be labeled as ^{64}Cu-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA)-scVEGF (^{64}Cu-DOTA-scVEGF), ^{99m}Tc-hydrazinonicotinic acid (HYNIC)-scVEGF (^{99m}Tc-HYNIC-scVEGF), and Cy5.5-scVEGF for imaging VEGFR expression to study tumor angiogenesis (10). ^{99m}Tc-HYNIC-scVEGF is being developed for single-photon emission computed tomography (SPECT) imaging of VEGFR-2 in tumor vasculature.

Synthesis

[PubMed]

Backer et al. (9) prepared a Cys-tagged vector of $VEGF_{121}$ by cloning two single-chain fragments (amino acid sequence 3–112) of $VEGF_{121}$ joining head-to-tail to express as scVEGF in *Escherichia coli*. ^{99m}Tc labeling of scVEGF was performed through HYNIC chelation (10). A mixture of scVEGF and HYNIC-maleimide in a molar ratio of 1:3 was incubated at room temperature for 60 min. The HYNIC-scVEGF conjugate was purified with column chromatography. The number of HYNIC molecules per protein was ~1. For radiolabeling, scVEGF was added to a ^{99m}Tc-tricine complex. The reaction mixture was incubated at 55°C for 1 h. ^{99m}Tc-HYNIC-scVEGF was purified with column chromatography. ^{99m}Tc-HYNIC-inVEGF, an inactive control, was prepared by conjugation of 7–8 biotins to ^{99m}Tc-HYNIC-scVEGF.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro competition binding studies were performed in 293/KDR cells expressing cloned human VEGFR-2 in competition with the chimeric toxin SLT-VEGF for binding to VEGFR-2 (10). ^{64}Cu-DOTA-PEG-scVEGF, DOTA-PEG-scVEGF, HYNIC-scVEGF, and Cy5.5-scVEGF inhibited the binding of SLT-VEGF to VEGFR-2. DOTA-PEG-scVEGF, HYNIC-scVEGF, and Cy5.5-scVEGF stimulated tyrosine phosphorylation of VEGFR-2 in 293/KDR cells in a manner similar to VEGF.

Animal Studies

Rodents

[PubMed]

Backer et al. (10) performed biodistribution studies of ^{99m}Tc-HYNIC-scVEGF and ^{99m}Tc-HYNIC-inVEGF in mice bearing 4T1 murine breast tumors expressing VEGFR-2. The tumor accumulated ~3% injected dose per gram (% ID/g), whereas the muscle accumulated <0.5% ID/g. The organs with the highest accumulation of ^{99m}Tc-HYNIC-scVEGF were the kidneys (~120% ID/g), lung (~6% ID/g), and liver (~6% ID/g) at 1 h after injection. ^{99m}Tc-HYNIC-scVEGF was cleared from the blood rapidly with ~2% ID/g at 50 min. ^{99m}Tc-HYNIC-scVEGF remained >95% intact at 3 and 50 min in the blood. PET imaging studies were performed at 1 h after injection of ^{99m}Tc-HYNIC-scVEGF or ^{99m}Tc-HYNIC-inVEGF. ^{99m}Tc-HYNIC-scVEGF revealed higher and more heterogeneous focal accumulation than ^{99m}Tc-HYNIC-inVEGF. Tumor/soft tissue ratio was 10–15 for ^{99m}Tc-HYNIC-scVEGF. Autoradiography of the tumor sections showed that ^{99m}Tc-HYNIC-scVEGF accumulated mainly at the outer rim of the tumor. No blocking experiments were performed.

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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