Microbubbles conjugated with single-chain Cys-tagged vascular endothelial growth factor-121

scVEGF-MBs

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Chemical name:	Microbubbles conjugated with single-chain Cys-tagged vascular endothelial growth factor-121	
Abbreviated name:	scVEGF-MBs	
Synonym:		
Agent Category:	Polypeptide	
Target:	Vascular endothelial growth factor receptor-2 (VEGFR-2)	
Target Category:	Receptor	
Method of detection:	Ultrasound	
Source of signal / contrast:	Microbubbles (MBs)	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about VEGF.

Background

[PubMed]

Ultrasound is the most widely used imaging modality (1) and its role in noninvasive molecular imaging is expanding with ligand-carrying microbubbles (MBs) (2). MBs are composed of spherical cavities filled by a gas encapsulated in a shell. The shells are made

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of phospholipids, surfactant, denatured human serum albumin, or synthetic polymer. Ligands and antibodies can be incorporated into the shell surface of MBs. MBs are usually 1–8 μm in diameter, and they provide a strongly reflective interface and resonate to ultrasound waves. MBs are used as ultrasound contrast agents in imaging of inflammation, angiogenesis, intravascular thrombus, and tumors (3-5). They also have the potential to be used for drug and gene delivery (6).

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 (7). $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$ are the forms secreted by most cell types, and they are active as homodimers linked by disulfide bonds. $VEGF_{121}$ does not bind to heparin like the other VEGF species (8). 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 (VEGFRs) 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 VEGFRs (7).

VEGFRs have been found to be overexpressed in various tumor cells and tumor-associated endothelial cells but are not detectable in quiescent endothelial cells (9). Inhibition of VEGFR function has been shown to inhibit pathological angiogenesis as well as tumor growth and metastasis (10, 11). Radiolabeled VEGF has been developed as a tracer for imaging solid tumors and angiogenesis in humans (12-14). MBs conjugated to either peptides or antibodies against integrins, cell adhesion molecules, and VEGFRs have previously been studied for the non-invasive assessment and imaging of tumor angiogenesis (15-18). A 15-amino-acid long fusion tag (Cys-tag) was developed for site-specific conjugation *via* the free sulfhydryl group of Cys. Backer et al. (19) prepared a Cys-tagged vector of $VEGF_{121}$ by cloning two single-chain 3–112 amino acid fragments of $VEGF_{121}$ joining head-to-tail to express as scVEGF, which binds to VEGFR-2. In this chapter, Anderson et al. (20) studied ultrasonic imaging of tumor vasculature using MBs conjugated with scVEGF (scVEGF-MBs) in mice bearing human tumor xenografts.

Related Resource Links:

- Chapters in MICAD (VEGFR)
- Gene information in NCBI (VEGFR)
- Articles in Online Mendelian Inheritance in Man (OMIM) (VEGFR)
- Clinical trials (VEGFR)
- FDA Drug information (VEGFR)

Synthesis

[PubMed]

Backer et al. (19) reported the synthesis of a Cys-tagged vector of $VEGF_{121}$ by cloning two single-chain 3–112 amino acid fragments of $VEGF_{121}$ joining head-to-tail to express

as scVEGF in *Escherichia coli* for mammalian cell production. Maleimide-bearing MBs (maleimide-MB) were reacted with a thio-containing scVEGF construct for 2 h at room temperature (20). scVEGF-MBs had a mean diameter of 2.5 ± 1.0 μm. There were 1.2×10^5 scVEGF molecules per MB.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Anderson et al. (20) reported that scVEGF-MBs or maleimide-MBs (5×10^6/ml) perfused for 5 min through flow chambers coated with VEGFR-2–positive porcine aortic endothelial cells at a wall shear rate of 1.0 dyne/cm^2. There was a significantly ($P < 0.01$) greater number of scVEGF-MBs attached to the endothelial cells (25 ± 4 MBs/cell) than maleimide-MBs (5 ± 1 MBs/cell). *In vitro* ultrasound studies were performed using flow assay with agar phantom, which was immobilized with murine VEGFR-2 or control casein. The mean pixel amplitude of adherent scVEGF-MBs (1×10^6/ml) was significantly higher ($P < 0.01$) in VEGFR-2–coated phantom (7 ± 2 dB) than in casein-coated phantom (0.2 dB).

Animal Studies

Rodents

[PubMed]

Anderson et al. (20) performed an ultrasound assessment of MB binding in mice bearing murine MC-38 colon adenocarcinoma tumors. Ultrasound was performed at 6 min after injection of scVEGF-MBs or maleimide-MBs (2×10^7/mouse). The mean pixel amplitudes in the tumor were 8.46 ± 1.61 dB and 1.58 ± 0.83 dB for scVEGF-MBs and maleimide-MBs, respectively. The contrast enhancement of scVEGF-MBs to the tumors was significantly higher than that of maleimide-MBs ($P < 0.01$). The tumor contrast induced by scVEGF-MBs returned to background level (~1 dB) when a high M1, destructive acoustic pulse was administered to the tumor tissues. However, no blocking studies were performed.

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.

NIH Support

1R43 EB007857, 2R44 EB007857, 2R44 CA113080

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