Microbubble-conjugated vascular endothelial growth factor receptor 2 monoclonal antibody

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Chemical name:	Microbubble-conjugated vascular endothelial growth factor receptor 2 monoclonal antibody	
Abbreviated name:	Microbubble-conjugated VEGFR2 mAb	
Synonym:	Ultrasound contrast agent (UCA) conjugated with an anti-VEGFR2 monoclonal antibody	
Agent Category:	Monoclonal antibody	
Target:	Vascular endothelial growth factor receptor 2 (VEGFR2)	
Target Category:	Antibody-ligand binding	
Method of detection:	Ultrasound	
Source of signal:	Microbubbles	
Activation:	No	
Studies:	• Rodents	Click here for the protein and mRNA sequence of human VEGFR.

Background

[PubMed]

There are three known receptors for the vascular endothelial growth factor (VEGF), designated as VEGF receptor 1 (VEGFR1), receptor 2 (VEGFR2), and receptor 3 (VEGFR3). During embryogenesis these receptors are involved in vasculogenesis, angiogenesis, and lymphangiogenesis (1, 2). Among the three receptors, VEGFR2 was shown to be the primary signal transducer for angiogenesis and the development of pathological conditions such as cancer and diabetic retinopathy. It has been shown that VEGFR2 is expressed mainly in the endothelial cells, and the expression is upregulated in

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the tumor vasculature (2-4). Thus the inhibition of VEGFR2 activity and its downstream signaling are important targets for the treatment of diseases involving angiogenesis. A variety of VEGFR2 antagonists, including kinase inhibitors and neutralizing antibodies, are now in clinical trials for the diagnosis and treatment of these pathologies. For such therapies to be successful, it is important to select patients who are most likely to respond to the treatment and to be able to rapidly evaluate the response after initiation of therapy. Resection of the tumor(s), followed by immunohistochemical analysis, is an invasive technique and may not always yield expected results because of sampling bias or errors (5, 6). Also, methods based on tumor size measurement by cross-sectional imaging are used to determine treatment response, but because of insensitivity of the method the size changes may not be detected, especially immediately after a treatment, or the effects may not be apparent because of a delayed response. This can lead to an inaccurate interpretation of results and conclusions, and the development of a suboptimal therapeutic regimen (7, 8).

Lyshchik et al. suggested that molecular ultrasonography could be a potential technique for the non-invasive investigation of tumor vasculature because the information on molecular and cellular profiles of the endothelial cells available with this technique can be correlated to data on the perfusion and microvascular blood volume obtained during the procedure (9). Microbubble (MB) ultrasound contrast agents (UCAs) conjugated to either peptides or antibodies against integrins have previously been used for the non-invasive assessment and imaging of angiogenesis (10, 11). However, because of the low-resolution images obtained during these studies, an accurate assessment of angiogenesis could not be made with these studies. Lyshchik et al. developed a UCA by conjugating a commercially available lyophilized MB preparation with biotinylated monoclonal antibodies (mAb) against VEGFR2 (9). The VEGFR2-conjugated UCA was used to investigate the expression of VEGFR2 on the vascular endothelium in a breast cancer murine model.

Synthesis

[PubMed]

The UCA was prepared with a commercially available MB kit specifically designed to conjugate the anti-VEGFR2 mAb, as recommended by the manufacturer (9). The UCA has streptavidin chemically linked to the phospholipid shell of the MB through a polyethylene glycol spacer and was designed to obtain maximum saturation of the MB surface with an antibody, leaving a minimal amount of free antibody in solution. The preparation was available to the investigators as a vial of lyophilized powder containing 9.2×10^8 dry, streptavidin-coated MBs that were filled with a mixture of nitrogen and perfluorobutane. No information regarding the number of antibody molecules linked to each MB were provided in the publication.

The dry UCA was resuspended in sterile saline and ~200 nmol of either biotinylated anti-mouse VEGFR2 mAb or immunoglobulin G isotype control were added to the solution to produce a VEGFR2-targeted UCA or a control UCA, respectively. The opacity of the UCA

was monitored visually to ensure no MB was destroyed. The storage conditions of the UCA after preparation, until used, were not provided by the investigators.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No references are currently available.

Animal Studies

Rodents

[PubMed]

The VEGFR2-targeted UCA was evaluated in athymic *nu*/*nu* mice. Mice (n = 5 in each group) were injected with murine 4T1 and 67NR breast cancer cells, respectively, in the hind limbs (9). The tumors were allowed to grow 6 to 8 days until they reached a maximum diameter of 5 to 8 mm. To reduce the chances of injection sequence bias, the mice were administered VEGFR2-targeted UCA or the control UCA in random order with a 30-min interval. The 4T1 tumors showed a moderate intensity signal of 15 ± 3.4 dB, and the control tumors had a mild signal intensity of 7 ± 1.6 dB. The 67NR tumors had a signal intensity of 50 ± 12.3 dB compared to the signal intensity of 12.0 ± 2.6 dB for the controls.

The degree of VEGFR2 expression on the two tumor types was also determined by immunoblotting and histochemistry (9). The 4T1 cell tumors exhibited a lower expression of VEGFR2 compared to the 67NR tumors. The vasculature of the two tumor types was also assessed by injecting fluoroscein isothiocynate-conjugated tomato lectin into the jugular vein of the mice 24 h after ultrasonography (9). The dye was allowed to circulate for 5 min, and then the mice were euthanized for resection of the tumors. This dye detects only functional blood vessels, and an increased number of blood vessels were observed in the 67NR tumors compared to those derived from the 4T1 cells. The investigators concluded that the ultrasound signal intensities observed with the tumors from the two cell lines correlated with the degree of VEGFR2 expression observed by immunoblotting and histochemistry.

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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