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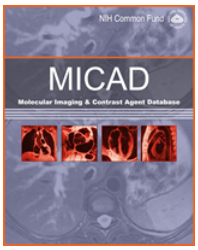

Microbubbles coated with biotinylated rabbit anti-mouse vascular endothelial growth factor receptor 2 (VEGFR-2) monoclonal antibody

MB_{vegfr2}

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Chemical name:	Microbubbles coated with rabbit anti-mouse vascular endothelial growth factor receptor 2 (VEGFR-2) monoclonal antibody	
Abbreviated name:	MB_{vegfr2}	
Synonym:		
Agent Category:	Antibody	
Target:	Vascular endothelial growth factor receptor 2 (VEGFR-2)	
Target Category:	Receptor	
Method of detection:	Ultrasound	
Source of signal / contrast:	Microbubbles	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Angiogenesis, the development of new vasculature from pre-existing blood vessels (for details see Carmeliet and Jain (1)), is essential for the development, maintenance, progression, and metastasis of neoplastic tumors (2). Therefore, angiogenesis is considered to be the hallmark of cancerous tumors, and early detection of this process can facilitate the initiation of treatment and management of the disease (3). Although there are several known pro-angiogenic biomarkers, among these only vascular endothelial growth factor receptor 2 (VEGFR-2) (4), $\alpha_V\beta_3$ integrin (5), and endoglin (6) are well characterized and are overexpressed in many cancerous tumors, such as those of the breast, ovaries, and the pancreas (3). The VEGFR-2 mediates its effects through a family of receptor tyrosine kinases that promote the mitogenesis, survival, differentiation, migration, and vascular permeability of endothelial cells (7). The $\alpha_V\beta_3$ integrins are heterodimeric cell adhesion molecules that can bind

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several different endogenous ligands such as fibronectin, von Willebrand factor, fibrinogen, etc., and assist with the survival and migration of cancerous cells, which increases the invasive potential of these cells, and promotes angiogenesis in tumors (8). Endoglin (CD105) is a co-receptor of the transforming growth factor beta (TGF-β) and co-modulates the different activities, including angiogenesis, of the activated TGF-β receptor (9).

Little information is available regarding the expression of the different angiogenic markers during the progression of a tumor from a small size to a larger size. Noninvasive visualization of angiogenic markers that are overexpressed during initial stages of the neoplasia can facilitate early detection and treatment of the disease (3). For this, Deshpande et al. developed a series of microbubble (MB; perfluorocarbon gas enclosed within spherical lipid shells harboring streptavidin moieties to bind biotinylated monoclonal antibodies (mAb) directed toward specific targets) based contrast agents that were coated with specific antibodies targeted to $\alpha_V\beta_3$ integrin VEGFR 2 (MB_{vegfr2}), ($MB_{integrin}$), and endoglin ($MB_{endoglin}$), respectively (3). The targeted MBs were then used with ultrasound imaging to determine the expression of the angiogenic biomarkers during the growth of human ovarian (SKOV3 cells), human breast (MDA-MB-361 cells), and human pancreatic (MiaPaCa2 cells) cell line xenograft tumors in mice. This chapter describes the studies performed with MB_{vegfr2}. Studies performed with $MB_{endoglin}$ and $MB_{integrin}$ are described in separate chapters of MICAD (www.micad.nih.gov) (10, 11).

Related Resource Links

VEGFR 2 related chapters in MICAD

Human VEGFR, variant 1, protein and mRNA sequences

Clinical trials related to VEGFR

VEGF and VEGFR signaling network (from Pathways Interaction Database)

United States Food and Drug Administration approved anti-VEGF drugs

Synthesis

[PubMed]

The synthesis of MB_{vegfr2} has been described in detail by Deshpande et al. (3). Briefly, the MBs were obtained as a freeze-dried preparation from a commercial source and reconstituted in 1 mL sterile 0.9% sodium chloride. Subsequently, 5×10^7 of the reconstituted MBs were incubated with 5 μg biotinylated anti-mouse VEGFR 2 mAb for 10 min at room temperature to obtain MB_{vegfr2}. A similar procedure was used to obtain $MB_{endoglin}$ and $MB_{integrin}$. For use as controls, another batch of MBs was linked to biotinylated rabbit immunoglobulin G antibodies ($MB_{control}$). The procedure used to remove excess mAb, if any, from the final preparations was not reported. The average number of antibody molecules bound per square micrometer of the MBs of each preparation was reported to be ~7,600 as determined with fluorescence-activated cell sorter analysis (3).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

A parallel plate flow chamber cell-binding assay with SVR cells (the high expression of VEGFR 2 and the other angiogenic biomarkers by these cells was confirmed with immunofluorescence staining) revealed that, compared to $MB_{control}$, a significantly higher ($P = 0.003$) amount of MB_{vegfr2} bound to these cells (3). Little or no binding of these MBs was observed with control cells (4T1 cells) that did not express any of the angiogenic biomarkers. An overall significantly positive correlation ($\rho = 0.83$; $P = 0.042$) was observed between the number of MBs that attached to the SVR cells and the expression of VEGFR 2 by the cells. Pre-incubation of the SVR cells with the rabbit anti-mouse VEGFR 2 mAb was reported to block the binding of MB_{vegfr2}.

An *ex vivo* immunofluorescence study of the tumor sections showed that VEGFR 2 in the cells was colocalized with CD31, a biomarker specific for endothelial cells (3). This indicated that the *in vivo* ultrasound signal obtained from the cancerous lesions was indeed generated by the MB_{vegfr2} bound to the VEGFR 2 receptors expressed by the cells in the tumors. In addition, a good correlation ($\rho = 0.63$) was evident between the *in vivo* ultrasound imaging signal and the *ex vivo* expression level of VEGFR 2 in the tumors ($P = 0.05$) (3).

Animal Studies

Rodents

[PubMed]

The expression of VEGFR 2 was investigated using MB_{vegfr2} with ultrasound imaging during the different growth size based stages (small, 50–150 mm^3; medium, 150–250 mm^3; large, >250 mm^3) of subcutaneous breast, ovarian, and pancreatic cell line xenograft tumors in mice ($n = 3$ animals/tumor type) as described by Deshpande at al (3). In addition, the expression of VEGFR-2 was compared with that of endoglin and $\alpha_V\beta_3$ integrin in these lesions.

Using the different MB based mAbs, ultrasound visualization of small and medium-sized breast cell line xenograft tumors in mice showed that the lesions expressed approximately the same levels ($P = 0.70$) of VEGFR-2 and $\alpha_V\beta_3$ integrin, but the expression of endoglin in the neoplasms was significantly higher ($P \leq 0.04$) than that of either VEGFR-2 or $\alpha_V\beta_3$ integrin (3). All the three angiogenic markers had a similar level of expression in the large tumors of the breast cell line ($P \geq 0.08$).

Ultrasound imaging with the different MB-antibody preparations showed that the expression of both VEGFR-2 and $\alpha_V\beta_3$ integrin was lower than that of endoglin ($P \leq 0.04$) in all sizes of the ovarian cell line xenograft tumors (3). No significant difference ($P \geq 0.40$) was observed in the expression level of VEGFR-2 and $\alpha_V\beta_3$ integrin in the different sizes of the ovarian cell line lesions.

The small pancreatic cell line xenograft tumors were shown to express lower levels of either VEGFR-2 ($P = 0.07$) or endoglin ($P = 0.15$) compared with $\alpha_V\beta_3$ integrin (3). No significant difference in the expression level of the three angiogenic markers was observed in the medium-sized tumors. Compared with VEGFR 2 ($P = 0.22$) the large pancreatic cell line lesions showed a significantly higher expression of endoglin ($P = 0.01$).

From these studies, the investigators concluded that the expression level of the different angiogenic markers varies with the growth stage of the tumor (3). They also concluded that targeted MB-based contrast agents can be used with ultrasound imaging to detect tumors in the early stages of growth in mice.

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.

Supplemental Information

[Disclaimers]

No Supplemental Information is currently available.

References

1. Carmeliet P., Jain R.K. *Molecular mechanisms and clinical applications of angiogenesis.* . Nature. 2011;473(7347):298–307. PubMed PMID: 21593862.
2. De Bock K., Mazzone M., Carmeliet P. *Antiangiogenic therapy, hypoxia, and metastasis: risky liaisons, or not?* . Nat Rev Clin Oncol. 2011;8(7):393–404. PubMed PMID: 21629216.
3. Deshpande N., Ren Y., Foygel K., Rosenberg J., Willmann J.K. *Tumor angiogenic marker expression levels during tumor growth: longitudinal assessment with molecularly targeted microbubbles and US imaging.* . Radiology. 2011;258(3):804–11. PubMed PMID: 21339349.
4. Shibuya M., Claesson-Welsh L. *Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis.* . Exp Cell Res. 2006;312(5):549–60. PubMed PMID: 16336962.
5. Cai W., Chen X. *Anti-angiogenic cancer therapy based on integrin alphavbeta3 antagonism.* . Anticancer Agents Med Chem. 2006;6(5):407–28. PubMed PMID: 17017851.
6. Nassiri F., Cusimano M.D., Scheithauer B.W., Rotondo F., Fazio A., Yousef G.M., Syro L.V., Kovacs K., Lloyd R.V. *Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy.* . Anticancer Res. 2011;31(6):2283–90. PubMed PMID: 21737653.
7. Koch S., Tugues S., Li X., Gualandi L., Claesson-Welsh L. *Signal transduction by vascular endothelial growth factor receptors.* . Biochem J. 2011;437(2):169–83. PubMed PMID: 21711246.
8. Wu G., Wang X., Deng G., Wu L., Ju S., Teng G., Yao Y., Liu N. *Novel peptide targeting integrin alphavbeta3-rich tumor cells by magnetic resonance imaging.* . J Magn Reson Imaging. 2011;34(2):395–402. PubMed PMID: 21780231.
9. Perez-Gomez E., Del Castillo G., Juan Francisco S., Lopez-Novoa J.M., Bernabeu C., Quintanilla M. *The role of the TGF-beta coreceptor endoglin in cancer.* . ScientificWorldJournal. 2010;10:2367–84. PubMed PMID: 21170488.
10. Chopra, A., *Microbubbles coated with biotinylated rabbit anti-mouse endoglin monoclonal antibody.* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.
11. Chopra, A., *Microbubbles coated with biotinylated rabbit anti-mouse alphaV beta3 integrin monoclonal antibody.* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -to current.