Microbubbles coated with biotinylated rabbit anti-mouse endoglin monoclonal antibody

$MB_{endoglin}$

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Chemical name:	Microbubbles coated with rabbit anti-mouse endoglin monoclonal antibody	
Abbreviated name:	$MB_{endoglin}$	
Synonym:		
Agent Category:	Antibody	
Target:	Endoglin	
Target Category:	Antigen	
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 endoglin (4), $\alpha_V\beta_3$ integrin (5), and the vascular endothelial growth factor receptor 2 (VEGFR-2) (6) are well

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characterized and are overexpressed in many cancerous tumors, such as those of the breast, ovaries, and the pancreas (3). 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 (7). The $\alpha_V\beta_3$ integrin is a G-protein–coupled receptor that can bind several different ligands such as fibronectin, von Willebrand factor, fibrinogen, etc., and assists with the survival and migration of cancerous cells, which increases the invasive potential of cancerous cells, and promotes angiogenesis in tumors (8). 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 (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, and the 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 endoglin ($MB_{endoglin}$), $\alpha_V\beta_3$ integrin ($MB_{integrin}$), and VEGFR 2 (MB_{vegfr2}), respectively (3). The targeted MBs were then used with ultrasound imaging to determine the expression of the angiogenic antigens 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 $MB_{endoglin}$. Studies performed with $MB_{integrin}$ and MB_{vegfr2} are described in separate chapters of MICAD (www.micad.nih.gov) (10, 11).

Related Resource Links

Microbubble related chapters in MICAD.

Homo sapiens endoglin transcript variant 1 protein and mRNA sequence.

Endoglin in Online Mendelian Inheritance in Man (OMIM) Database.

Endoglin in Gene Expression Omnibus (GEO) Database

Synthesis

[PubMed]

The synthesis of $MB_{endoglin}$ 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 endoglin mAb for 10 min at room temperature to obtain $MB_{endoglin}$. A similar procedure was used to obtain $MB_{integrin}$ and MB_{vegfr2}. 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 preparation was not reported. The average number of antibody molecules bound per square micrometer of the MBs 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 endoglin 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 $MB_{endoglin}$ 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 endoglin by the cells. Pre-incubation of the SVR cells with the rabbit anti-mouse endoglin mAb was reported to block the binding of the $MB_{endoglin}$.

An *ex vivo* immunofluorescence study of the tumor sections showed that endoglin 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 lesion was indeed generated by the $MB_{endoglin}$ bound to the antigen expressed by the cells in the tumors. In addition, a good correlation ($\rho = 0.88$) was evident between the *in vivo* ultrasound imaging signal and the *ex vivo* expression level of endoglin in the tumors ($P = 0.002$) (3).

Animal Studies

Rodents

[PubMed]

The expression of endoglin was investigated using $MB_{endoglin}$ with ultrasound imaging during the different stages based on growth size (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 endoglin was compared with that of $\alpha_V\beta_3$ integrin and VEGFR-2 in these lesions.

The expression of endoglin was reported to be significantly higher ($P \leq 0.04$) than that of $\alpha_V\beta_3$ integrin and VEGFR-2 in the small and medium-sized breast cell line xenograft tumors in the mice (3). The expression levels of $\alpha_V\beta_3$ integrin and VEGFR-2 in these lesions were approximately the same ($P = 0.70$), and the large tumors expressed similar levels of all the three antigens ($P \geq 0.08$).

The expression level of endoglin in all sizes of the ovarian cell line xenograft tumors, as determined with ultrasound imaging using $MB_{endoglin}$, was reported to be higher than

that of either $\alpha_V\beta_3$ integrin or VEGFR-2 ($P \leq 0.04$) (3). No significant difference ($P \geq 0.40$) was observed in the expression level of $\alpha_V\beta_3$ integrin and VEGFR-2 in all sizes of the xenograft tumors.

In the pancreatic cell line xenograft tumors, the small lesions expressed higher levels of $\alpha_V\beta_3$ integrin compared with either endoglin ($P = 0.15$) or VEGFR-2 ($P = 0.07$) (3). No significant difference in the expression level of the three antigens in the medium-sized tumors was observed. The large pancreatic lesions showed a significantly higher ($P = 0.01$) expression of endoglin compared with VEGFR2 but not $\alpha_V\beta_3$ integrin ($P = 0.22$).

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.

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