

Microbubbles coated with biotinylated rabbit anti-mouse $\alpha v \beta 3$ integrin monoclonal antibody

MB_{integrin}

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Chemical name:	Microbubbles coated with rabbit anti-mouse $\alpha v \beta 3$ integrin monoclonal antibody	
Abbreviated name:	MB _{integrin}	
Synonym:		
Agent Category:	Antibody	
Target:	$\alpha v \beta 3$ integrin	
Target Category:	Receptor	
Method of detection:	Ultrasound	
Source of signal / contrast:	Microbubbles	
Activation:	No	
Studies:	<ul style="list-style-type: none">• <i>In vitro</i>• 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 $\alpha v \beta 3$ integrin (4), endoglin (5), and the vascular endothelial growth factor receptor 2 (VEGFR-2) (6) are

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well characterized and are overexpressed in many cancerous tumors, such as those of the breast, ovaries, and the pancreas (3). The $\alpha_V\beta_3$ integrins are heterodimeric cell adhesion molecules that can bind 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 (7). Endoglin (CD105) is a co-receptor of the transforming growth factor beta (TGF- β) and co-modulates different activities, including angiogenesis, of the activated TGF- β receptor (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. 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 (MB_{integrin}), endoglin (MB_{endoglin}), and VEGFR 2 (MB_{vegfr2}), 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_{integrin}. Studies performed with MB_{endoglin} and MB_{vegfr2} are described in separate chapters of MICAD (www.micad.nih.gov) (10, 11).

Related Resource Links

Related chapters in [MICAD](#)

Homo sapiens integrin, alpha V, transcript variant 1, [protein and mRNA sequence](#)

Rat (*Rattus norvegicus*) integrin, beta 3, [protein and mRNA sequence](#)

[Clinical trials](#) related to integrins

Integrin alpha V in [Online Mendelian Inheritance in Man Database \(OMIM\)](#)

Integrin beta 3 in [Online Mendelian Inheritance in Man Database \(OMIM\)](#)

[Integrin signaling pathway](#) in [Pathway Interaction Database](#)

Synthesis

[[PubMed](#)]

The synthesis of MB_{integrin} 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 μ g biotinylated anti-mouse α ν β ₃ integrin mAb for 10 min at room temperature to obtain MB_{integrin}. A similar procedure was used to obtain MB_{endoglin} 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 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 α ν β ₃ integrins 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_{integrin} 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 ($\rho = 0.87$; $P = 0.019$) was observed between the number of MBs that attached to the SVR cells and the expression of α ν β ₃ integrins by the cells. Pre-incubation of the SVR cells with the rabbit anti-mouse α ν β ₃ integrin mAb was reported to block the binding of MB_{integrin}.

An *ex vivo* immunofluorescence study of the tumor sections showed that α ν β ₃ integrins 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_{integrin} bound to the α ν β ₃ integrin receptors expressed by the cells in the tumors. In addition, a good correlation ($\rho = 0.65$) was evident between the *in vivo* ultrasound imaging signal and the *ex vivo* expression level of α ν β ₃ integrins in the tumors ($P = 0.05$) (3).

Animal Studies

Rodents

[PubMed]

The expression of α ν β ₃ integrins was investigated using MB_{integrin} with ultrasound imaging during the different growth stages based on tumor size (small, 50–150 mm³; medium, 150–250 mm³; large, >250 mm³) of subcutaneous breast, ovarian, and pancreatic cell line xenograft tumors in mice ($n = 3$ animals/tumor type) as described by Deshpande et al (3). In addition, the expression of α ν β ₃ integrin was compared with that of endoglin and VEGFR-2 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 $\alpha_V\beta_3$ integrin and VEGFR-2, but the expression of endoglin in the neoplasm was significantly higher ($P \leq 0.04$) than that of $\alpha_V\beta_3$ integrin and VEGFR-2 (3). A similar level of all the three angiogenic markers was observed in 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 $\alpha_V\beta_3$ integrin and VEGFR-2 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 $\alpha_V\beta_3$ integrin and VEGFR-2 in the different sizes of the ovarian cell line lesions.

The small pancreatic cell line xenograft tumors were shown to express 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 angiogenic markers in the medium-sized pancreatic cell line tumors was observed. The large pancreatic lesions showed a significantly higher ($P = 0.01$) expression of endoglin compared with VEGFR2 ($P = 0.22$).

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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