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Supporting Information

Multimodal Interventional Molecular Imaging of Tumor Margins and Distant Metastases by Targeting $\alpha_v\beta_3$ Integrin

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Supporting Information

Flow cytometry analysis of cell binding

Freshly cultured MDAMB231^{CXCR4+} or GEβ3 cells (increased $\alpha_v\beta_3$ expression) were trypsinized, aliquoted in portions of 300.000 cells, centrifuged (1200 rpm, 5 min, 4 °C) and decanted. 5 nM of monoclonal phycoerythrin (PE) labelled anti-integrin β_3 antibody (C17 (37.5 μg/mL), ab91128, Abcam, Cambridge, UK) or 0.1 μM of RGD-MSAP in 50 μL of 0.1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) was added to the cells. After 1 hour of incubation at 4 °C, the cells were washed (2 ×) with 300 μL of 0.1% BSA in PBS, resuspended in 300 μL of 0.1% BSA in PBS and fluorescence was measured using a CyAn ADP flow cytometer (DakoCytomation) with PE settings (488 nm laser and 575/25 nm filter) for antibody or APC-Cy7 settings (635 nm laser and 750 nm long pass filter) for RGD-MSAP. Live cells were gated on Forward Scatter, Side Scatter and Pulse Width and approximately 10.000 viable cells were analyzed. All experiments were performed in triplicate. Mean Fluorescence Intensity Ratios (MFIR) were calculated by dividing the mean fluorescence signals from incubated cells by the background fluorescence signal from not incubated cells. The ratio of MFIR between GEβ3 and MDAMB231^{CXCR4+} were calculated by dividing the MFIR of the GEβ_3 cells by the MFIR of the MDAMB231^{CXCR4+}.

Biodistribution ^{99m}Tc-Vasculosis

One vial of Vasculosis (Iba, Belgium; 10 mg, 150 nmole) was dissolved in 1.5 mL of 0.9% saline to which 0.5 mL $^{99m}TcO_4^-$ (710 MBq) was added. After one hour incubation 0.2 mL of the solution was injected iv in the tail vein of balb/c nude mice (n=3). The animals were sacrificed 24 hours post injection and tissue samples and organs were removed. The biodistribution of ^{99m}Tc-Vasculosis was determined by measuring the amount of radioactivity present in the organs, using a gamma counter (Wizard 3" 1480 automatic gamma counter, Perkin Elmer). Counts per minute were converted into MBq and corrected for decay. The percentage of the injected dose per gram of tissue (%ID/g) was calculated as followed: ((MBq measured in tissue/ injected dose) *100%)/weight of tissue.

Autofluorescence signal of tissues

We determined the autofluorescence originating from different tissue samples from mice (n=5), which were not injected with an imaging agent, and of the standard chow used to feed the mice (irradiated AM-II chow; Abdiets #2146). The tissues were arranged on a black paper background to minimize reflections and the autofluorescence was measured at the Cy5.5$_{695\text{-}770nm}$ settings of the IVIS 200. The fluorescent signals measured for the different tissues were divided by the background (the underlying black paper from the same image) to obtain an autofluorescence to background ratio (ABR). A ratio of 1 means no autofluorescence compared to the background. Ratios below one are caused by tissues with less reflectance than the background.

Results and discussion

Flow cytometry

As determined by binding of the PE-labeled anti integrin β3 antibody, MDAMB231 cells show no detectable membrane expression of the $\alpha_v\beta_3$ integrin, while the GEβ3 can be successfully labeled by the fluorescently labeled antibody yielding a mean fluorescence intensity ratio (MFIR) of 17.1. The RGD-MSAP demonstrated also binding to the $\alpha_v\beta_3$ integrin expressing GEβ3 cells, yielding a similar pattern as the labeling with the antibody. This demonstrates that this RGD-derivative has retained his affinity for the $\alpha_v\beta_3$ integrin. However, incubation of the MDAMB231 cells with RGD-MSAP showed some aspecific binding of the probe. Taking this into account resulted in a MFIR of 4.1.

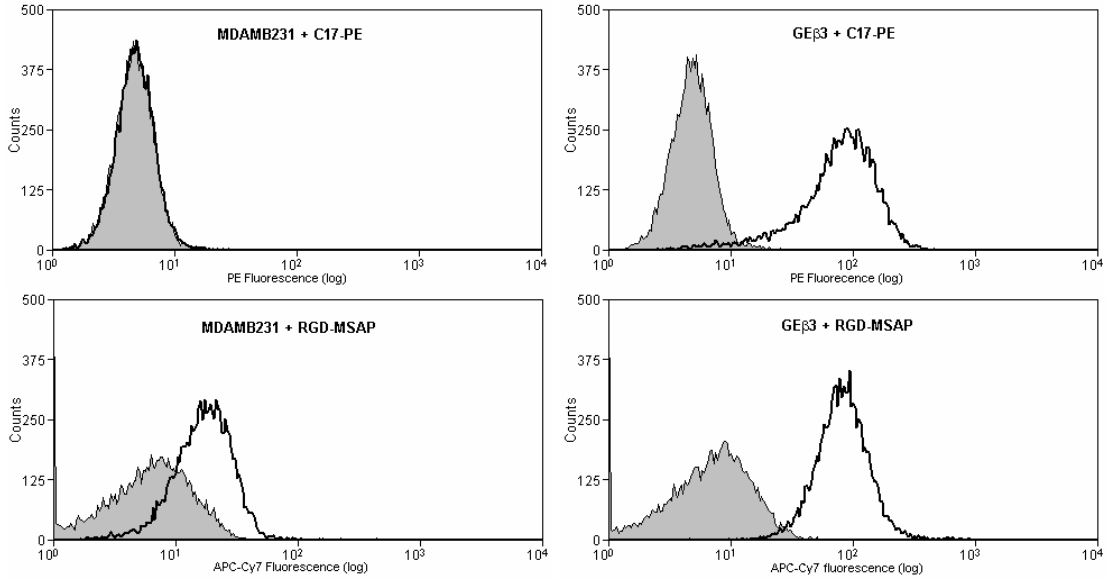

Figure SI1 Flow cytometric analysis of the binding of a PE-labeled anti-integrin β_3 antibody and RGD-MSAP to MDAMB231 and GEβ3 cells.

Autofluorescence signal

One possible drawback of the use of fluorescence imaging for biodistribution studies in vivo is the autofluorescence of tissues caused by fluorophores present in tissues, blood or chow. From figure S2 it becomes clear that most tissues do not or only slightly show autofluorescence at the excitation and emission wavelengths of Cy5.5. The kidneys and liver show moderate autofluorescence. In the stomach and intestines the autofluorescence signal was much higher. These two organs contain the non-specialized chow the animals were given, which is highly autofluorescent.

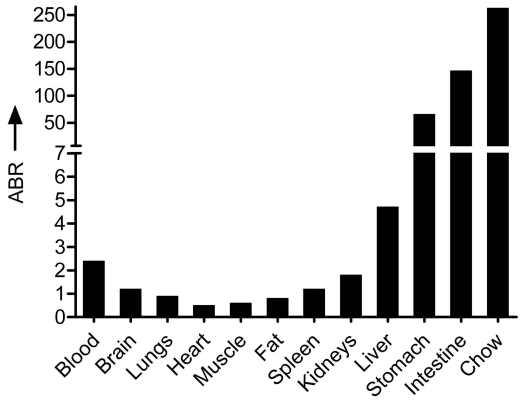

Figure SI2 Autofluorescence of mouse organs and normal chow (Cy5.5 settings: λ_{ex}=615-665nm, λ_{em}=695-770nm) given as autofluorescence to background ratio (ABR).

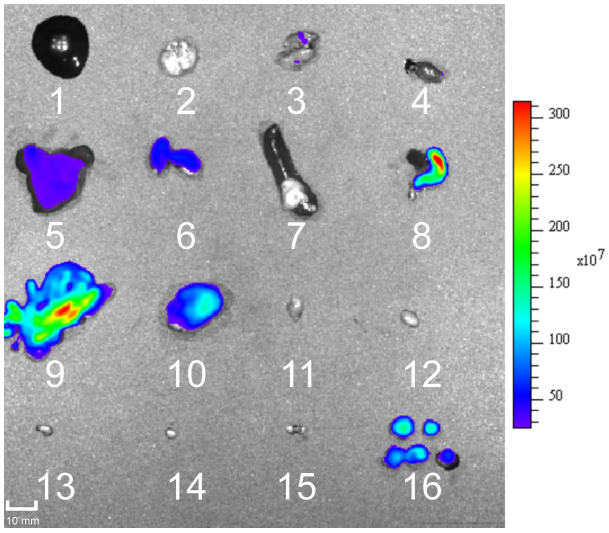

Figure SI3 *Ex vivo* fluorescence imaging of the organs of the mouse from figure 3 (blood (1), brain (2), lung (3), heart (4), Liver (5), kidneys (6), spleen (7), stomach (8), intestines (9), primary tumor (10), muscle (11), mammary fat pad (12), LNs (13-15) and distant metastases (16).).

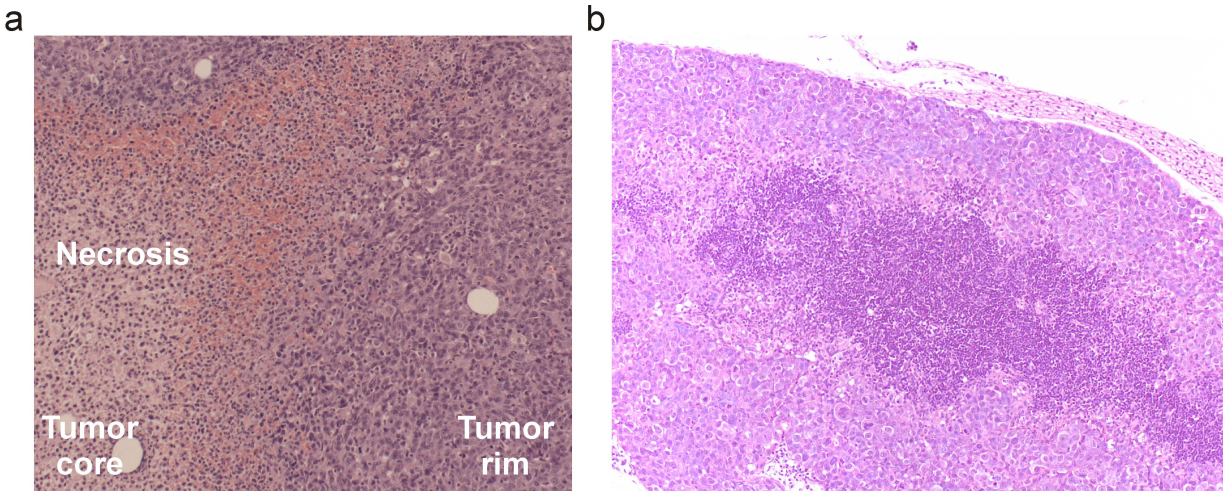

Figure SI4 A) Transition of the viable rim of the tumor to the necrotic core visualized with standard H&E staining, B) Pathologic analysis of an excised metastases containing tumor cells.