D-Cys-D-Asp-Gly-Tyr(3-NO_2)-Gly-ProOH-Asn-D-Cys-biotin-streptavidin-Cy5.5

LXY2-biotin-SA-Cy5.5

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Chemical name:	D-Cys-D-Asp-Gly-Tyr(3-NO_2)-Gly-ProOH-Asn-D-Cys-biotin-streptavidin-Cy5.5	
Abbreviated name:	LXY2-biotin-SA-Cy5.5	
Synonym:	cdGT(3-NO_2)GBNc-biotin-SA-Cy5.5	
Agent category:	Peptide	
Target:	Integrin α_3	
Target category:	Receptor	
Method of detection:	Optical, near-infrared (NIR) fluorescence imaging	
Source of signal:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about integrin α_3.

Background

[PubMed]

Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets small animals (1, 2). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-infrared (NIR) fluorescence (700–1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between

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target and background tissues. NIR fluorophores have a wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a non-invasive alternative to radionuclide imaging.

Integrins are a family of cell surface heterodimeric glycoproteins that mediate diverse biological events involving cell–cell and cell–matrix interactions (3). They consist of an α and a β subunit, and they are important for cell adhesion and signal transduction. The $\alpha_3\beta_1$ integrin plays an important role in normal lung, kidney, cerebral cortical, and epithelial development (4). On the other hand, it affects tumor growth, tumor invasiveness, and metastasis, as the α_3 integrin is strongly expressed on tumor cells (5, 6). D-Cys-D-Asp-Gly-Leu-Gly-ProOH-Asn-D-Cys (LXY1), a cyclic peptide, was identified to bind to α_3 integrin on human ovarian cancer cells using "one-bead one compound" combinatorial libraries (7, 8). LXY1 was conjugated with Cy5.5 *via* biotin-streptavidin (SA) to study *in vivo* biodistribution of the tracer in tumor-bearing mice. LXY1-biotin-SA-Cy5.5 was found to have a high specific accumulation in α_3-positive human glioblastoma U-87MG cells in nude mice (9). Another LXY1 analog, D-Cys-D-Asp-Gly-Tyr(3-NO_2)-Gly-ProOH-Asn-D-Cys (LXY2), was identified to exhibit a higher binding affinity than LXY1 (10). LXY2 was conjugated with Cy5.5 *via* biotin-SA to study *in vivo* biodistribution of the tracer in tumor-bearing mice. LXY2-biotin-SA-Cy5.5 was found to have a high specific accumulation in α_3-positve human breast adenocarcinoma MDA-MB-231 cells in nude mice. Cy5.5 is a NIR fluorescence dye with an absorbance maximum at 675 nm and an emission maximum at 694 nm with a high extinction coefficient of 250,000 $M^{-1}cm^{-1}$.

Synthesis

[PubMed]

LXY2 and LXY2-biotin were constructed using solid-phase synthesis (10). SA-Cy5.5 (1.8 nmol) and LXY2-biotin (7.2 nmol) were incubated for 18 h at 4°C to form LXY2-biotin-SA-Cy5.5 (molecular weight, 64 kDa) complexes with a 4:1 molar ratio of biotin/SA.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Xiao et al. (9) showed that the binding affinity (K_d) of LXY1-biotin for integrin α_3 on U-87MG cells was 0.5 ± 0.1 μM. The LXY1-biotin binding was completely inhibited by excess LXY1 and anti-α_3 antibody. Yao et al. (10) showed that LXY1-biotin bound only to integrin α_3 and not to other integrins tested (α_1, α_2, α_3, α_5, α_6, α_9, α_V, β_1, β_2, β_3, β_4, and β_5) and had a K_d value of 0.4 ± 0.1 μM for integrin α_3 on MDA-MB-231 cells. Using the MDA-MB-231 binding assay, IC_{50} values of LXY1 and LXY2 were 550 ± 0.17 and 57

± 0.01 nM, respectively. The binding of LXY2-biotin on the MDA-MB-231 cells was inhibited by LXY2 and anti-α_3 antibody.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of LXY2-biotin-SA-Cy5.5 were evaluated in nude mice bearing subcutaneous MDA-MB-231 and K562 (α_3-negatve) xenografts (10). *Ex vivo* images were obtained after injection of 7.2 nmol LXY1-biotin-SA-Cy5.5 at 6 h after injection. The NIR fluorescence intensities of LXY2-biotin-SA-Cy5.5 in the MDA-MB-231 tumor, liver, and kidneys were high, with low NIR fluorescence intensities in the K562 tumors, skin, intestine, spleen, and muscle. Pretreatment with 0.13 nmol anti-α_3 integrin antibody 1 h before administration of LXY2-biotin-SA-Cy5.5 showed that accumulation of the imaging probe in the MDA-MB-231 tumor was almost completely blocked by the anti-α3 integrin antibody. On the other hand, the pretreatments had no effect on the NIR fluorescence signals in the liver and kidneys. Whole-body NIR fluorescence imaging showed that the MDA-MB-231 tumor showed a peak NIR fluorescence signal at 6 h and slowly washed out. However, the tumor was still clearly visualized at 72 h.

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.

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