D-Cys-D-Asp-Gly-Leu-Gly-ProOH-Asn-D-Cys-biotin-streptavidin-Cy5.5

LXY1-biotin-SA-Cy5.5

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Chemical name:	D-Cys-D-Asp-Gly-Leu-Gly-ProOH-Asn-D-Cys-biotin-streptavidin-Cy5.5	
Abbreviated name:	LXY1-biotin-SA-Cy5.5	
Synonym:	cdGLGBNc-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 in 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 target and background tissues. NIR fluorophores have a wider dynamic range and

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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 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. 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 $M^{-1}cm^{-1}$. LXY1-Biotin-SA-Cy5.5 was found to have a high specific accumulation in α_3-positve human glioblastoma U-87MG cells in nude mice (9).

Synthesis

[PubMed]

LXY1 and LXY1-biotin were constructed using solid-phase synthesis (9). SA-Cy5.5 (1.8 nmol) and LXY1-biotin (7.2 nmol) were incubated for 18 h at 4°C to form LXY1-biotin-SA-Cy5.5 (molecular weight, 64 kDa) complexes with a 4:1 molar ratio of biotin/SA. LXY1-Lys(NH_2)-$CONH_2$ was incubated with Cy5.5-NHS for 18 h to form LXY1-Lys-Cy5.5 (LXY1-Cy5.5). The NHS ester of Cy5.5 was reacted with the ε-amino group of the lysine. The peak containing the LXY1-Cy5.5 conjugate (2.28 kDa) was analyzed with matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) and found to be consistent with 1 Cy5.5 per LXY1.

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 to U-87MG cells was completely inhibited by excess LXY1 and anti-α_3 antibody. Yao et al. (10) showed that LXY1 binds 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).

Animal Studies

Rodents

[PubMed]

Biodistribution studies of LXY1-biotin-SA-Cy5.5 were evaluated in nude mice (n = 3/group) bearing subcutaneous U-87MG and K562 (α_3-negatve) xenografts (9). *Ex vivo* images were obtained after injection of 7.2 nmol LXY1-biotin-SA-Cy5.5 at 0.5, 4, 6, 24, and 48 h after injection. The NIR fluorescence intensities of LXY1-biotin-SA-Cy5.5 in the U-87MG tumor and kidneys peaked at ~4 h and then decreased gradually, but >80% of the maximum value was retained in the tumor even at 48 h. The NIR fluorescence intensities in the liver, skin, intestine, spleen, and brain were lower than the intensities in the tumor and kidneys. Little NIR fluorescence signal was observed in the K562 tumors. Pretreatment with 3.6 μmol LXY1 or 0.13 nmol anti-α_3 integrin antibody 1 h before administration of LXY1-biotin-SA-Cy5.5 showed that accumulation of the imaging probe in the U-87MG tumor was almost completely blocked by LXY1 ($P < 0.001$) and anti-α3 integrin antibody ($P < 0.001$). On the other hand, the pretreatments had no effect on the NIR fluorescence signals in the liver and kidneys. The smaller univalent LXY1-Cy5.5 exhibited a faster accumulation in the tumor and kidneys, and LXY1-Cy5.5 exhibited a more rapid clearance than the multivalent LXY1-biotin-SA-Cy5.5. Whole-body NIR fluorescence imaging showed that the U-87MG tumor but not the K562 tumor was clearly visualized with LXY1-biotin-SA-Cy5.5.

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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