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NLM Citation: Leung K. D-Cys-D-Asp-Gly-HCit-Gly-Pro-Gln-D-Cys-Ebes-Lys-polyethylene glycol-cholic acids-Cy5.5 telodendrimer nanoparticles. 2012 Jun 1 [Updated 2012 Sep 7]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
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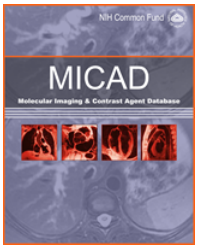

D-Cys-D-Asp-Gly-HCit-Gly-Pro-Gln-D-Cys-Ebes-Lys-polyethylene glycol-cholic acids-Cy5.5 telodendrimer nanoparticles

OA02-PEG^{5k}-CA$_8$-Cy5.5 NPs

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Created: June 1, 2012; Updated: September 7, 2012.

Chemical name:	D-Cys-D-Asp-Gly-HCit-Gly-Pro-Gln-D-Cys-Ebes-Lys-polyethylene glycol-cholic acids-Cy5.5 telodendrimer nanoparticles	
Abbreviated name:	OA02-PEG^{5k}-CA$_8$-Cy5.5 NPs	
Synonym:		
Agent category:	Peptide	
Target:	Integrin α_3	
Target category:	Receptor	
Method of detection:	Optical, Near-Infrared	
Source of signal:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about integrin $\alpha_3\beta_1$.

Background

[PubMed]

Integrins are a family of cell-surface heterodimeric glycoproteins that mediate diverse biological events involving cell–cell and cell–matrix interactions (1). They consist of an α and a β subunit. 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 (2). On the other hand, it affects tumor growth, tumor invasiveness, and metastasis as the $\alpha_3\beta_1$ integrin is strongly expressed on tumor cells (3, 4). D-Cys-D-Asp-Gly-HCit-Gly-Pro-Gln-D-Cys (OA02) was identified to bind to the α_3 integrin on human ovarian cancer cells using one-bead-one-compound combinatorial libraries (5, 6). OA02 was conjugated with Cy5.5 to study *in vivo* biodistribution of the tracer in tumor-bearing mice (7). Cy5.5 is a NIR fluorescent dye with an absorbance maximum at 675 nm and

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emission maximum at 694 nm with a high extinction coefficient of 250,000 $(mol/L)^{-1}cm^{-1}$. OA02-Cy5.5 was found to have a high specific accumulation in $\alpha_3\beta_1$-positve ES-2 human ovarian tumor cells in nude mice.

Polymeric micelles compose of a core-shell structure formed by amphiphilic block copolymers (telodendrimers) with nanoparticle sizes of 20-60 nm (8). Ligands and labels can be conjugated to the shell surface and drugs can be encapsulated inside the core of micelles. Xiao et al. (9) conjugated OA02 to micelles comprising linear copolymers of polyethylene glycol (PEG) and cholic acids (CA). Cy5.5 was conjugated to the amino group of the proximal Lys between PEG and cholic acid (CA) to form D-Cys-D-Asp-Gly-HCit-Gly-Pro-Gln-D-Cys-Ebes-Lys-PEG^{5k}-CA_8-Cy5.5 telodendrimer nanoparticles (OA02-PEG^{5k}-CA_8_Cy5.5 NPs) for *in vivo* near-infrared (NIR) fluorescence imaging of α_3_expressing SKOV-3 human ovarian tumor xenografts in nude mice.

Related Resource Links:

- Chapters in MICAD (OA02)
- Gene information in NCBI (α_3 integrin, β_1 integrin)
- Articles in Online Mendelian Inheritance in Man (OMIM) (α_3 integrin, β_1 integrin)

Synthesis

[PubMed]

Alkyne modified OA02 peptide (D-Cys-D-Asp-Gly-HCit-Gly-Pro-Gln-D-Cys-Ebes-Lys-alkyne) was prepared using solid-phase peptide synthesis and conjugated to the N_3-PEG^{5k}-CA_8 telodendrimer monomer via Cu-catalyzed cyloaddition to form OA02-PEG^{5k}-CA_8 telodendrimer monomer (9). There was one OA02 peptide per telodendrimer monomer (9.9 kDa) as measured with MALDI-TOF mass spectroscopy. Cy5.5 monofunctional *N*-hydroxysuccinimide (NHS) ester was used to conjugate OA02-PEG^{5k}-CA_8 telodendrimer monomer. The NHS ester of Cy5.5 reacted with the amino group of the proximal Lys between PEG and CA. PEG^{5k}-CA_8 and OA02-PEG^{5k}-CA_8-Cy5.5 telodendrimer monomers were mixed in chloroform in 1:1 molar ratio. After evaporation of chloroform, the residue film was dissolved in phosphate-buffered saline to form OA02-PEG^{5k}-CA_8_Cy5.5 NPs. Non-targeted PEG^{5k}-CA_8_Cy5.5 NPs were also prepared. The number of Cy5.5 and OA02 molecules per NP was not reported. The particle sizes were 20.5 ± 1.9 nm and 21.0 ± 2.3 for OA02-PEG^{5k}-CA_8_Cy5.5 NPs and PEG^{5k}-CA_8_Cy5.5 NPs, respectively. OA02-PEG^{5k}-CA_8_FITC NPs and PEG^{5k}-CA_8_FITC NPs were prepared similarly for *in vitro* studies.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Receptor-mediated endocytosis of OA02-PEG^{5k}-CA_8_FITC NPs in SKOV-3 human ovarian tumor cells (α_3-positive) and K562 human erythroleukemia cells (α_3-negative) was observed by fluorescence confocal microscopy and flow cytometry (9). The fluorescence staining of OA02-PEG^{5k}-CA_8_FITC NPs (2 μM) was confined to the cell membrane and cytoplasm after 2 h of incubation at 37°C. Free OA02 peptide (200 μM) and excess anti-α_3 antibody inhibited the uptake of OA02-PEG^{5k}-CA_8_FITC NPs in SKOV-3 cells. On the other hand, K562 cells showed minimal uptake of OA02-PEG^{5k}-CA_8_FITC NPs. Flow cytometry analysis showed that SKOV-3 cells exhibited 5-fold higher uptake of OA02-PEG^{5k}-CA_8_FITC NPs than PEG^{5k}-CA_8_FITC NPs. The uptake was inhibited by >70% with 100-fold excess OA02 peptide. Furthermore, uptake of non-targeted NPs and targeted NPs was low and similar in K562 cells. Both NPs were not cytotoxic to SKOV-3 cells after 2 h of incubation.

Animal Studies

Rodents

[PubMed]

Whole-body NIR fluorescence imaging studies of OA02-PEG^{5k}-CA$_8$-Cy5.5 NPs or PEG^{5k}-CA$_8$-Cy5.5 NPs (0.4 nmol Cy5.5) were evaluated in nude mice (n = 3/group) bearing SKOV-3 subcutaneous xenografts after intravenous injection (9). Both NPs were distributed throughout the body of the mice at 10 min after injection. Tumor accumulation of OA02-PEG^{5k}-CA$_8$-Cy5.5 NPs was apparent at 2 h with clear visualization at 4 h. Tumor retention remained throughout the 24 h of imaging period. On the other hand, tumor accumulation of PEG^{5k}-CA$_8$-Cy5.5 NPs was not apparent until 8 h after injection with a decrease of fluorescence intensity by 24 h. *Ex vivo* biodistribution studies of OA02-PEG^{5k}-CA$_8$-Cy5.5 NPs and PEG^{5k}-CA$_8$-Cy5.5 NPs were performed at 24 after injection. The tumor accumulation (mean fluorescence intensity, MFI) of OA02-PEG^{5k}-CA$_8$-Cy5.5 NPs (4,400 AU) was ~70% higher ($P < 0.05$) than that of PEG^{5k}-CA$_8$-Cy5.5 NPs (2,600 AU). The accumulations in the normal organs were similar for both tracers with the highest accumulations in the liver (3,000 AU) and urinary bladder (2,500 AU). The muscle exhibited a low background level with MFI value of 1,000 AU. Histo-immunologic analysis of tumor sections showed that OA02-PEG^{5k}-CA$_8$-Cy5.5 NPs co-localized with vascular endothelial cells (CD31-positive and α_3-positive) and SKOV-3 tumor cells (α_3-positive), whereas PEG^{5k}-CA$_8$-Cy5.5 NPs remained mainly in the perivascular region. PEG^{5k}-CA$_8$-Cy5.5 NPs were able to extravasate from the tumor vasculatures to bind to the tumor cells. Blocking studies were not performed.

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.

NIH Support

R01 CA140449, R01 CA115483, R01 EB012569

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