Gly-Ser-Ser-Lys-(FITC)-Gly-Gly-Gly-Cys-Arg-Gly-Asp-Cys-CLIO-Cy5.5

cRGD-CLIO(Cy5.5)

Huiming Zhang, PhD[1]

Created: December 17, 2007; Updated: January 18, 2008.

Chemical name:	Gly-Ser-Ser-Lys-(FITC)-Gly-Gly-Gly-Cys-Arg-Gly-Asp-Cys-CLIO-Cy5.5	
Abbreviated name:	cRGD-CLIO(Cy5.5)	
Synonym:		
Agent category:	Peptide, small molecule (nanoparticle)	
Target:	$\alpha_v\beta_3$ integrin	
Target category:	Receptor	
Method of detection:	Magnetic resonance imaging (MRI), fluorescence molecular tomography (FMT), fluorescence reflectance imaging (FRI)	
Source of signal/contrast:	Iron oxides, Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

The $\alpha_v\beta_3$ integrin, also known as the vitronectin receptor, is a heterodimeric transmembrane glycoprotein found on most cells originating from mesenchyme (1). This receptor is often overexpressed in various tumor cells, including osteosarcomas, neuroblastomas, glioblastomas, invasive melanomas, and carcinomas of the lung, breast, prostate, and bladder (1). Many extracellular matrix proteins such as fibronectin,

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Zhang H. Gly-Ser-Ser-Lys-(FITC)-Gly-Gly-Gly-Cys-Arg-Gly-Asp-Cys-CLIO-Cy5.5. 2007 Dec 17 [Updated 2008 Jan 18]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

vitronectin, thrombospondin, fibrinogen, osteopontin, and tenascin are known to be involved in interactions with various subtypes of integrins (1). These proteins may contain a variety of motifs for potential cell binding; however, one of the most frequent cell-recognition motifs includes an amino acid sequence of Arg-Gly-Asp (RGD), called "the universal cell-recognition site" (1) or "a versatile cell recognition signal" (2). The binding potency of the RGD motif leads to the development of small homing peptides, whose high affinity to the $\alpha_v\beta_3$ integrin provides a promising alternative to antibodies in targeting tumors (3). As a result, RGD analogs are widely used in tumor imaging, anti-angiogenesis treatment, and tumor-associated radionucleotides or chemotherapeutic drugs. Some RGD analogs are currently being used in phase II clinical trials (4). These $\alpha_v\beta_3$ integrin–specific probes will help oncologists improve the delineation of tumors and follow up the progression of anti-angiogenic therapies (4).

Gly-Ser-Ser-Lys-(FITC)-Gly-Gly-Gly-Cys-Arg-Gly-Asp-Cys-cross-linked iron oxide-Cy5.5 (cRGD-CLIO(Cy5.5)) is a magneto-fluorescent nanoparticle for multimodal imaging of $\alpha_v\beta_3$ integrin. This agent consists of three components: an RGD peptide for targeting $\alpha_v\beta_3$ integrin, two fluorescence probes for optical detection/imaging, and an iron oxide nanoparticle core for magnetic resonance imaging (MRI) contrast enhancement (5). The peptide contains 12 amino acids with an intramolecular disulfide bond to form a cyclic RGD (cRGD). One of the fluorescence probes, fluorescein isothiocyanate (FITC), is attached to the peptide before conjugation to the nanoparticle, allowing for the characterization of peptide/iron ratio and the quantification of cell-associated peptide or peptide-nanoparticle on the basis of the absorption at 493 nm. The other fluorescence probes (i.e., Cy5.5 or Cy3.5) are cyanine dyes consisting of two quaternized heteroaromatic bases (A and A') joined by a polymethine chain with five (Cy5.5) or three (Cy3.5) carbons (6) and directly bound to the nanoparticle (5). These dyes have a cationic character because of the delocalized positive charge of the chromophore, and they possess high quantum yield, good chemical stability, easy conjugation, and high sensitivity (mole extinction coefficient ~ 250,000 mol/cm) (7, 8). The excitation/emission wavelength is 674/692 nm for Cy5.5 and 548/563 nm for Cy3.5, where hemoglobin and water have their lowest absorption coefficient. The difference in wavelength of Cy5.5/Cy3.5 allows for dual wavelength ratio imaging to quantify the components labeled with Cy5.5 or Cy3.5 (9).

The nanoparticle contains an icosahedral core of superparamagnetic crystalline Fe_3O_4 (magnetite) (10) that is caged by epichlorohydrin cross-linked dextran and functionalized with amine groups (CLIO-NH_2) (11). These amino groups are used in further conjugation chemistry for attaching the RGD peptides and the fluorescent dyes. The superparamagnetic crystalline includes a sufficiently large single-domain of unpaired spins to generate a net magnetic moment that is larger than the sum of its individual unpaired electrons (10, 12). The main difference between these superparamagnetic nanoparticles and paramagnetic ions such as gadolinium (Gd) is their large magnetic moment unhindered by lattice orientation (13). Thus, they possess a high magnetic susceptibility that results in a significant induced magnetization inside a magnetic field. This, in turn, creates microscopic field gradients that diphase nearby protons and cause T_2

shortening (13). CLIO-NH_2 has a magnetite core of ~5 nm with a hydrodynamic diameter of 20 nm (11, 12), which can lead to a three- to four-fold increase in T_1 relaxivity and a five- to six-fold increase in T_2 relaxivity compared to conventional contrast agents such as Gd-diethylenetriamine pentaacetic acid (Gd-DTPA) (10). CLIO-NH_2 is suitable for receptor-directed MRI or magnetically labeled cell probe MRI because it is small enough to easily pass through capillary endothelium while retaining superparamagnetism (12). Despite its small size, CLIO still exhibits superparamagnetic properties and is detectable at tissue concentrations of only 50 nmol Fe/g tissue ($10^{13–14}$ iron particles/g tissue) (10).

Synthesis

[PubMed]

Montet et al. reported a detailed synthesis of cRGD-CLIO(Cy5.5) (5). Initially, a linear RGD peptide GSSK(FI)GGGCRGDC (IRGD) was obtained with the Fmoc method as a C-terminal amide and oxidized *via* bubbling air to yield a disulfide-linked cRGD peptide in 0.1 M ammonium bicarbonate. The amino-CLIO nanoparticle was synthesized in several steps. The starting material, monocrystalline iron oxide (MION), was synthesized by neutralization of ferrous salts, ferric salts, and dextran with ammonium hydroxide, followed by ultra-filtration (14). The obtained MION was cross-linked in strong base with epichlorohydrin and then reacted with ammonia to produce amino-CLIO (CLIO-NH_2) (14). Finally, CLIO-NH_2 was reacted with the N-hydroxysuccinimide ester of Cy5.5 (Amersham Biosciences Corp., Piscataway, NJ), followed by peptide attachment using disuccinimidyl suberimidate to produce cRGD-CLIO(Cy5.5) (5). There were ~250 amines, 8,000 iron atoms, 27 peptides, and 8 Cy5.5 molecules per CLIO-NH_2 nanoparticle.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The uptake of cRGD-CLIO(Cy5.5) was examined in human breast carcinomas (BT-20) by iron stain or Cy5.5 fluorescence (5). After injection of nanoparticles, iron and fluorescence were broadly distributed through the tumor. The molecular specificity for cRGD-CLIO(Cy5.5) binding to $\alpha_v\beta_3$ integrin was determined in BT-20 cells (5). First, the 50% effective concentration (EC_{50}) of cRGD-CLIO(Cy5.5) was compared with that of IRGD-CLIO(Cy5.5) when they were bound to the BT-20 cells. The EC_{50} was found to be 0.0113 μM for the cyclic isomer and 0.4 μM for the linear isomer using a fluorescence-activated cell-sorting (FACS) cytometer. This demonstrated that the cRGD had a 35-fold increase in affinity compared to the linear RGD. Second, the binding affinity of cRGD-CLIO(Cy5.5) was compared with that of a scrambled peptide analog (srcRGD-CLIO(Cy3.5)) by measuring their uptake in the BT-20 tumor. Animals with tumors were euthanized 24 h after intravenous injection of mixed cRGD-CLIO(Cy5.5) and srcRGD-CLIO(Cy3.5) at 5 mg Fe/kg dose, and slices of tissues were imaged with a multichannel

fluorescent imager. The Cy5.5/Cy3.5 ratio was found to be 6.5 in the BT-20 tumor and ~1 in the liver or spleen, where a high concentration of nanoparticles accumulated. The distribution of cRGD-CLIO(Cy5.5) in the BT-20 tumor was illustrated with iron staining and fluorescence microscopy, in which both iron and Cy5.5 fluorescence were observed throughout the whole tumor.

The expression of $\alpha_v\beta_3$ integrin in rat gliosarcomas (9L) was first examined with cRGD alone by fluorescein immunoassay (5). The apparent affinity constant of cRGD-CLIO in the 9L cells was very similar to that in the BT-20 cells, but the maximum amount (B_{max}) of cRGD bound to $\alpha_v\beta_3$ integrin was 0.16 pmol in the 9L cells, which is about four times lower than the 0.82 pmol in the BT-20 cells. This indicated that expression of the $\alpha_v\beta_3$ integrin in the 9L tumor was four times less than that in the BT-20 tumors. Then, the binding affinity of cRGD-CLIO(Cy5.5) in the 9L tumors was compared with that of srcRGD-CLIO(Cy3.5). The Cy5.5/Cy3.5 fluorescence ratio was 1.8, which was 3.6 times lower than that in the BT-20 tumor. In addition, the cRGD-CLIO(Cy5.5) was found to have a hydrodynamic diameter of 28 ± 3 nm with a T_2 relaxivity of 111 $mM^{-1}s^{-1}$ at 4.7 T (5).

Animal Studies

Rodents

[PubMed]

cRGD-CLIO (Cy5.5) was used to examine the expression of $\alpha_v\beta_3$ integrin in BT-20 tumors (3–4 mm in diameter) implanted in nude mice. Fluorescence reflectance imaging (FRI) was conducted after intravenous injection of cRGD-CLIO(Cy5.5)/srcRGD-CLIO(Cy3.5) at 5 mg Fe/kg. FRI showed a much higher tumor/background ratio at the Cy5.5 channel than at the Cy3.5 channel during the first 1,500 min after injection. Because the blood half-life time of cRGD-CLIO(Cy5.5) was 180 mins, the signal enhancement in the Cy5.5 channel reflected the accumulation of cRGD-CLIO(Cy5.5) in the tumor cells. Fluorescence molecular tomography (FMT) and MRI at 4.7 T were performed after intravenous injection of 3 mg Fe/kg cRGD-CLIO(Cy5.5). FMT images demonstrated different enhancements for deep tumor slices. MRI images exhibited an apparent T_2-shortening effect caused by cRGD-CLIO(Cy5.5) because the mean tumor T_2 dropped from 77 ms before injection to 66 ms 24 h after injection.

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

P50 CA86355, R24 CA92782, R01 EB00662

References

1. Haubner R., Finsinger D., Kessler H. Stereoisomeric peptide libaries and peptidomimetics for designing selective inhibitors of the aub3 integrin for a new cancer therapy. Angew. Chem. Int. Ed. Engl. 1997;36:1375–1389.
2. Ruoslahti E., Pierschbacher M.D. Arg-Gly-Asp: a versatile cell recognition signal. Cell. 1986;44(4):517–8. PubMed PMID: 2418980.
3. Zitzmann S., Ehemann V., Schwab M. Arginine-glycine-aspartic acid (RGD)-peptide binds to both tumor and tumor-endothelial cells in vivo. Cancer Res. 2002;62(18): 5139–43. PubMed PMID: 12234975.
4. Garanger E., Boturyn D., Dumy P. Tumor targeting with RGD peptide ligands-design of new molecular conjugates for imaging and therapy of cancers. Anticancer Agents Med Chem. 2007;7(5):552–8. PubMed PMID: 17896915.
5. Montet X., Montet-Abou K., Reynolds F., Weissleder R., Josephson L. Nanoparticle imaging of integrins on tumor cells. Neoplasia. 2006;8(3):214–22. PubMed PMID: 16611415.
6. Ernst L.A., Gupta R.K., Mujumdar R.B., Waggoner A.S. Cyanine dye labeling reagents for sulfhydryl groups. Cytometry. 1989;10(1):3–10. PubMed PMID: 2917472.
7. Lin Y., Weissleder R., Tung C.H. Novel near-infrared cyanine fluorochromes: synthesis, properties, and bioconjugation. Bioconjug Chem. 2002;13(3):605–10. PubMed PMID: 12009952.
8. Ballou B., Fisher G.W., Hakala T.R., Farkas D.L. Tumor detection and visualization using cyanine fluorochrome-labeled antibodies. Biotechnol Prog. 1997;13(5):649–58. PubMed PMID: 9336985.
9. Kircher M.F., Josephson L., Weissleder R. Ratio imaging of enzyme activity using dual wavelength optical reporters. Mol Imaging. 2002;1(2):89–95. PubMed PMID: 12920849.
10. Shen T., Weissleder R., Papisov M., Bogdanov A. Jr, Brady T.J. Monocrystalline iron oxide nanocompounds (MION): physicochemical properties. Magn Reson Med. 1993;29(5):599–604. PubMed PMID: 8505895.
11. Josephson L., Perez J.M., Weissleder R. Magnetic nanosensors for the detection of oligonucleotide sequences. Angew. Chem. Int. Ed. Engl. 2001;40:3204–06. PubMed PMID: 29712059.

12. Wang Y.X., Hussain S.M., Krestin G.P. Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. Eur Radiol. 2001;11(11):2319–31. PubMed PMID: 11702180.
13. Bulte J.W., Brooks R.A., Moskowitz B.M., Bryant L.H. Jr, Frank J.A. T1 and T2 relaxometry of monocrystalline iron oxide nanoparticles (MION-46L): theory and experiment. Acad Radiol. 1998;5Suppl 1S137–40. PubMed PMID: 9561064.
14. Wunderbaldinger P., Josephson L., Weissleder R. Crosslinked iron oxides (CLIO): a new platform for the development of targeted MR contrast agents. Acad Radiol. 2002;9Suppl 2S304–6. PubMed PMID: 12188255.