Cy5.5-Cyclo(CGNSNPKSC)

Cy5.5-GX1

Kam Leung, PhD[1]

Created: January 30, 2012; Updated: May 3, 2012.

Chemical name:	Cy5.5-Cyclo(CGNSNPKSC)	
Abbreviated name:	Cy5.5-GX1	
Synonym:		
Agent category:	Peptide	
Target:	Tumor vasculature	
Target category:	Receptor	
Method of detection:	Optical, near-infrared (NIR) fluorescence	
Source of signal:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Angiogenesis is an essential process in the development of new blood vessels in normal physiological states and in diseases (1, 2). Targeting of tumor vasculature is a promising strategy for tumor imaging and therapy because tumor growth and metastasis largely depend on angiogenesis (3, 4). A cyclic 9-mer peptide (CGNSNPKSC (GX1)) was initially identified as a recognition motif for binding to the human gastric tumor vasculature (5-7). GX1 has also been shown to bind to a variety of human cancer vasculatures. The molecular target of GX1 has not been identified; however, GX1 inhibits endothelial cell proliferation *in vitro* and neovascularization *in vivo*. The Lys (K) residue of GX1 was conjugated with Cy5.5 to study *in vivo* biodistribution of the tracer in mice bearing

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

Corresponding author.

NLM Citation: Leung K. Cy5.5-Cyclo(CGNSNPKSC). 2012 Jan 30 [Updated 2012 May 3]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

U89MG human glioblastoma tumors (8). Cy5.5-GX1 exhibited high near-infrared (NIR) fluorescence intensity in U89MG tumors in nude mice.

Related Resource Links:

- Chapters in MICAD (GX1)

Synthesis

[PubMed]

The Boc-protected GX1 peptide was obtained using solid-phase synthesis (8). Cy5.5-*N*-hydroxysuccinimide (NHS) ester (500 nmol) was used to conjugate the peptide (500 nmol) for 2 h at 45°C. The NHS ester of Cy5.5 reacted with the Ɛ-amino group of the Lys residue of the peptide. The Boc-protected Cy5.5-GX1 was isolated with high-performance liquid chromatography (HPLC), with 66% yield. Trifluoracetic acid was used to remove the Boc-protecting group to form Cy5.5-GX1, with 76% yield and >97% chemical purity after HPLC purification. Mass spectroscopy analysis confirmed a 1:1 addition of Cy5.5 to GX1. Fluorescence excitation and emission maxima of Cy5.5 were 675 nm and 695 nm, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Flow cytometry analysis showed that 6%, 12%, and 25% of U89MG cells were positive after incubation with 0.5, 1, and 4 nmol Cy5.5-GX1 for 60 min at 4°C (8). Confocal microscopy showed internalization of fluorescence activity inside the cells. Neither binding affinity of the derivatized peptide for the tumor vasculature nor blocking studies were performed with unlabeled GX1.

Animal Studies

Rodents

[PubMed]

Chen et al. (8) performed NIR fluorescence imaging of nude mice ($n = 3$) bearing U89MG tumors at 0.5–24 h after intravenous injection of 1 nmol Cy5.5-GX1. The tumor accumulation of Cy5.5-GX1 could be clearly visualized from 1 h to 24 h, with a maximum contrast at 4–8 h. The tumor/background ratio was ~4 at 8 h after injection. Co-injection of GX1 (20 μmol/kg) inhibited the fluorescence signal to near background level at 4 h after injection ($P < 0.05$). *Ex vivo* NIR fluorescence imaging of tissues at 24 h after injection showed that the tissues with the highest signals (photons/s/cm^2/steradian) were the tumor (1.77×10^8) and liver (0.44×10^8), followed by the kidney (0.22×10^8), lung ($<0.2 \times 10^8$), spleen ($<0.2 \times 10^8$), heart ($<0.2 \times 10^8$), blood ($<0.2 \times 10^8$), and muscle ($<0.2 \times 10^8$). The tumor/muscle, tumor/liver, and tumor/kidney ratios were 15.21 ± 0.84, 4.02

± 0.13, and 7.98 ± 0.44, respectively, whereas the corresponding values for the blocked group were 6.95 ± 0.75, 2.79 ± 0.35, and 4.67 ± 0.55, respectively.

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.

References

1. Carmeliet P. *Manipulating angiogenesis in medicine.* J Intern Med. 2004;255(5):538–61. PubMed PMID: 15078497.
2. Folkman J. *Angiogenesis: an organizing principle for drug discovery?* Nat Rev Drug Discov. 2007;6(4):273–86. PubMed PMID: 17396134.
3. Abdollahi A., Folkman J. *Evading tumor evasion: current concepts and perspectives of anti-angiogenic cancer therapy.* Drug Resist Updat. 2010;13(1-2):16–28. PubMed PMID: 20061178.
4. Thorpe P.E. *Vascular targeting agents as cancer therapeutics.* Clin Cancer Res. 2004;10(2):415–27. PubMed PMID: 14760060.
5. Chen B., Cao S., Zhang Y., Wang X., Liu J., Hui X., Wan Y., Du W., Wang L., Wu K., Fan D. *A novel peptide (GX1) homing to gastric cancer vasculature inhibits angiogenesis and cooperates with TNF alpha in anti-tumor therapy.* BMC Cell Biol. 2009;10:63. PubMed PMID: 19740430.
6. Hui X., Han Y., Liang S., Liu Z., Liu J., Hong L., Zhao L., He L., Cao S., Chen B., Yan K., Jin B., Chai N., Wang J., Wu K., Fan D. *Specific targeting of the vasculature of gastric cancer by a new tumor-homing peptide CGNSNPKSC.* J Control Release. 2008;131(2): 86–93. PubMed PMID: 18700158.
7. Zhi M., Wu K.C., Dong L., Hao Z.M., Deng T.Z., Hong L., Liang S.H., Zhao P.T., Qiao T.D., Wang Y., Xu X., Fan D.M. *Characterization of a specific phage-displayed Peptide binding to vasculature of human gastric cancer.* Cancer Biol Ther. 2004;3(12):1232–5. PubMed PMID: 15492500.
8. Chen, K., L.P. Yap, R. Park, X. Hui, K. Wu, D. Fan, X. Chen, and P.S. Conti, *A Cy5.5-labeled phage-displayed peptide probe for near-infrared fluorescence imaging of tumor vasculature in living mice.* Amino Acids, 2011