Cyclo(Cys-Asn-Gly-Arg-Cys)-Gly-Lys-Cy5.5

Cy5.5-cNGR

Kam Leung, PhD[1]

Created: January 15, 2009; Updated: March 19, 2009.

Chemical name:	Cyclo(Cys-Asn-Gly-Arg-Cys)-Gly-Lys-Cy5.5	
Abbreviated name:	Cy5.5-cNGR	
Synonym:		
Agent category:	Peptide	
Target:	Aminopeptidase N (CD13)	
Target category:	Enzyme	
Method of detection:	Optical, near-infrared (NIR) fluorescence imaging	
Source of signal/contrast:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Extracellular matrix (ECM) adhesion molecules consist of a complex network of fibronectins, collagens, chondroitins, laminins, glycoproteins, heparin sulfate, tenascins, and proteoglycans that surround connective tissue cells, and they are mainly secreted by fibroblasts, chondroblasts, and osteoblasts (1). Cell substrate adhesion molecules are considered essential regulators of cell migration, differentiation, and tissue integrity and remodeling. These molecules play a role in inflammation and atherogenesis, but they also participate in the process of invasion and metastasis of malignant cells in the host tissue (2). Invasive tumor cells adhere to the ECM, which provides a matrix environment for permeation of tumor cells through the basal lamina and underlying interstitial stroma of

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

Corresponding author.

NLM Citation: Leung K. Cyclo(Cys-Asn-Gly-Arg-Cys)-Gly-Lys-Cy5.5. 2009 Jan 15 [Updated 2009 Mar 19]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

the connective tissue. Overexpression of matrix metalloproteinases (MMPs) and other proteases by tumor cells allows intravasation of tumor cells into the circulatory system after degrading the basement membrane and ECM (3).

Tumor angiogenesis represents a continuous and important process in tumor development in which the tumor attempts to gain an independent blood supply (4). This process is driven by the tumor's overproduction of angiogenic factors, which bind to receptors on nearby vessel endothelial cells. Angiogenesis is essential for the growth of solid tumors and their metastases. Imaging angiogenesis may be useful for monitoring angiogenic treatments of tumors and cardiovascular diseases (5-7). Aminopeptidase N (APN, CD13) is a membrane bound glycoprotein with MMP activity that cleaves unsubstituted, N-terminal amino acids with neutral side chains from peptides (8). APN has been shown to play a role in tumor angiogenesis, invasion, and metastasis (9). In addition to endothelial cells of angiogenic vessels, most cells of myeloid origin, epithelial cells, fibroblasts and smooth muscle cells also express CD13 (10, 11). The tumor homing peptide cyclo(Cys-Asn-Gly-Arg-Cys)-Gly-Lys (cNGR) contains the Asn-Gly-Arg (NGR) motif that binds to APN (12). von Wallbrunn et al. (13) prepared Cy5.5-labeled cNGR (Cy5.5-cNGR) for imaging APN expression in tumors.

Synthesis

[PubMed]

Cy5.5 Monofunctional *N*-hydroxysuccinimide ester was used to conjugate cNGR to form Cy5.5-cNGR, which was purified with high-performance liquid chromatography with >95% purity (13). Cy5.5-cNGR was adjusted to a final concentration of 2 mg/ml. A molar ratio of Cy5.5 to cNGR was estimated to be ~1.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Histoimmunostaining of CD13 was performed on HT-1080 and MCF-7 cancer cell lines (13). The APN enzyme was expressed on the cell surface of HT-1080 tumor cells, whereas expression in MCF-7 tumor cells was only background staining. The APN activity in HT-1080 cells could be blocked with anti-CD13 antibodies and the cNGR peptide.

Animal Studies

Rodents

[PubMed]

von Wallbrunn et al. (13) performed *ex vivo* biodistribution studies of Cy5.5-cNGR in nude mice (n = 6) bearing HT-1080 fibrosarcoma tumors. Near-infrared fluorescence was measured in tumor, muscle, heart, lung, spleen, kidney, and liver at 24 h after injection of

2 nmol Cy5.5-cNGR. The organ with the highest concentration was the kidney (793 ± 272 AU), followed by the liver (398 ± 87 AU), tumor (332 ± 152 AU), and lung (307 ± 39 AU). Pretreatment with cNGR (10 mg/kg) 10 min before Cy5.5-cNGR injection resulted in decreased uptake in all dissected tissues. A similar distribution pattern was found in mice (n = 5) bearing MCF-7 tumors except lower fluorescence signal in the MCF-7 tumors (220 ± 10 AU) and kidney (500 ± 50 AU). Three-dimensional fluorescence-mediated tomography were also performed in mice bearing the tumor xenografts, showing an average Cy5.5-cNGR concentration of 307 ± 54 nM Cy5.5 (HT-1080) and 116 ± 18 nM Cy5.5 (MCF-7) in the tumors after 5 h. Pretreatment with cNGR (10 mg/kg) resulted in a reduction of tracer concentration in HT-1080 tumor tissue (195 ± 22 nM) at 5 h after injection. The tumor/background ratio was 7.5 ± 4.0 for Cy5.5-cNGR alone and 3.3 ± 2.1 for Cy5.5-cNGR with cNGR blocking. Histoimmunostaining of CD13 was performed on HT-1080 and MCF-7 tumor sections at 24 h after injection of Cy5.5-cNGR. The APN enzyme was expressed in HT-1080 tumor cells with intense membrane Cy5.5 fluorescence; whereas MCF-7 tumor cells exhibited marginal APN expression and Cy5.5 signal.

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. Bosman F.T., Stamenkovic I. *Functional structure and composition of the extracellular matrix.* J Pathol. 2003;200(4):423–8. PubMed PMID: 12845610.
2. Jiang W.G., Puntis M.C., Hallett M.B. *Molecular and cellular basis of cancer invasion and metastasis: implications for treatment.* Br J Surg. 1994;81(11):1576–90. PubMed PMID: 7827878.
3. Albelda S.M. *Role of integrins and other cell adhesion molecules in tumor progression and metastasis.* Lab Invest. 1993;68(1):4–17. PubMed PMID: 8423675.
4. Folkman J. *Angiogenesis in cancer, vascular, rheumatoid and other disease.* Nat Med. 1995;1(1):27–31. PubMed PMID: 7584949.

5. Sinusas A.J. *Imaging of angiogenesis.* J Nucl Cardiol. 2004;11(5):617–33. PubMed PMID: 15472646.
6. Carmeliet P. *Manipulating angiogenesis in medicine.* J Intern Med. 2004;255(5):538–61. PubMed PMID: 15078497.
7. Miller J.C., Pien H.H., Sahani D., Sorensen A.G., Thrall J.H. *Imaging angiogenesis: applications and potential for drug development.* J Natl Cancer Inst. 2005;97(3):172–87. PubMed PMID: 15687360.
8. Riemann D., Kehlen A., Langner J. *CD13--not just a marker in leukemia typing.* Immunol Today. 1999;20(2):83–8. PubMed PMID: 10098327.
9. Sato Y. *Role of aminopeptidase in angiogenesis.* Biol Pharm Bull. 2004;27(6):772–6. PubMed PMID: 15187415.
10. Corti A., Curnis F., Arap W., Pasqualini R. *The neovasculature homing motif NGR: more than meets the eye.* Blood. 2008;112(7):2628–35. PubMed PMID: 18574027.
11. Curnis F., Arrigoni G., Sacchi A., Fischetti L., Arap W., Pasqualini R., Corti A. *Differential binding of drugs containing the NGR motif to CD13 isoforms in tumor vessels, epithelia, and myeloid cells.* Cancer Res. 2002;62(3):867–74. PubMed PMID: 11830545.
12. Arap W., Pasqualini R., Ruoslahti E. *Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model.* Science. 1998;279(5349):377–80. PubMed PMID: 9430587.
13. von Wallbrunn A., Waldeck J., Holtke C., Zuhlsdorf M., Mesters R., Heindel W., Schafers M., Bremer C. *In vivo optical imaging of CD13/APN-expression in tumor xenografts.* J Biomed Opt. 2008;13(1):011007. PubMed PMID: 18315356.