IPLVVPLGGSC(Cy5.5-Cross-linked iron oxide)K(Fitc)

IPL-NP

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Chemical name:	IPLVVPLGGSC(Cy5.5-Cross-linked iron oxide)K(Fitc)	
Abbreviated name:	IPL-NP	
Synonym:		
Agent Category:	Peptide	
Target:	Hepsin	
Target Category:	Enzyme binding	
Method of detection:	Optical, near-infrared fluorescence,	
Source of signal\contrast:	Cy5.5, iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Optical fluorescence imaging is increasingly used to understand biological functions of specific targets (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 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,

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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 in small animals.

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 (3). 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 (4). 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 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 (5).

Hepsin is a type II transmembrane serine protease that is generally not detectable in normal and benign hypertrophic prostate glands. Hepsin is also expressed in the liver, kidney, and thyroid (6). Elevated levels of hepsin have been found in prostate tumors (7). Hepsin promotes prostate cancer progression and metastasis (8). The peptide IPLVVPL was found to bind selectively to hepsin from phage library screening. Kelly et al. (9) used this sequence with linking residues GGSCK(Fitc) with Cy5.5-cross-linked iron oxide nanoparticles (Cy5.5-CLIO) to attach to the IPLVVPL to form fluorescence nanoparticles, IPLVVPLGGSC(Cy5.5-CLIO)K(Fitc). Cy5.5 is a NIR fluorescent dye with an absorbance maximum at 675 nm and an emission maximum at 694 nm with a high extinction coefficient of 250,000 $M^{-1}cm^{-1}$. IPLVVPLGGSC(Cy5.5-CLIO)K(Fitc) is being developed for NIR fluorescence imaging of hepsin expression in prostate cancer.

Synthesis

[PubMed]

Aminated Cy5.5-CLIO was incubated with excess succinimidyl iodoacetic acid for 15 min (9). IPLVVPLGGSCK(Fitc) was coupled to the activated CLIO *via* a thiol-specific reaction at the C-terminal amino acid (C) for 1 h. The resulting IPLVVPLGGSC(Cy5.5-CLIO)K(Fitc) (IPL-NP) was purified with column chromatography. Each CLIO backbone contained an average of 11 peptides.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Kelly et al. (9) performed cell-binding assays (analyzed with flow cytometry and fluorescence microscopy) with IPL molecules using prostate cancer cell lines expressing hepsin (HPN-PC3 and LNCaP) as well as prostate cancer cell lines that do not express

hepsin (PC3 and DU145). IPLVVPL-Fitc and IPLVVPLGGSCK(Fitc) exhibited good binding to HPN-PC3 and LNCaP cells with minimal binding to PC3 and DU145 cells. IPLVVPL inhibited IPLVVPLGGSCK(Fitc) binding to HPN-PC3 cells with a 50% inhibition constant of 1.4 ± 1.7 μM and a dissociation constant of 190 ± 2.2 nM. IPL-NP exhibited a 10-fold greater fluorescence intensity than IPLVVPLGGSCK(Fitc) in binding to HPN-PC3 cells and a >80-fold increase in fluorescence intensity in binding to NPN-PC3 cells as compared with PC3 cells. *In situ* fluorescent histochemical analysis of frozen human tissue samples of six normal prostate and five prostate cancers revealed that IPLVVPLGGSCK(Fitc) and IPL-NP stained 100% of the tumor cores (localized to tumor cells) and 0% of the control prostate cores.

Animal Studies

Rodents

[PubMed]

Kelly et al. (9) performed *in vivo* IPL-NP fluorescence-mediated tomography imaging of mice bearing an LNCaP tumor on the right flank and a PC3 tumor on the left flank. Increased fluorescence signal could be detected in the LNCaP tumor (65 ± 3 nM) 24 h after injection of 20 mg Fe/kg, whereas the PC3 tumor showed a lower signal (23 ± 1 nM) at the same time point. The blood half-life of IPL-NP was determined to be 7.4 h. Control non-conjugated nanoparticle accumulation in both tumors was ~20 nM. LNCaP tumors as small as 4.6 mm in diameter could be detected. No blocking or co-localization experiments were 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.

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References

1. Ntziachristos V., Bremer C., Weissleder R. Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging. Eur Radiol. 2003;**13**(1):195–208. PubMed PMID: 12541130.
2. Achilefu S. Lighting up tumors with receptor-specific optical molecular probes. Technol Cancer Res Treat. 2004;**3**(4):393–409. PubMed PMID: 15270591.
3. Bosman F.T., Stamenkovic I. Functional structure and composition of the extracellular matrix. J Pathol. 2003;**200**(4):423–8. PubMed PMID: 12845610.
4. 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.
5. 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.
6. Wu Q., Parry G. Hepsin and prostate cancer. Front Biosci. 2007;**12**:5052–9. PubMed PMID: 17569629.
7. Stephan C., Yousef G.M., Scorilas A., Jung K., Jung M., Kristiansen G., Hauptmann S., Kishi T., Nakamura T., Loening S.A., Diamandis E.P. Hepsin is highly over expressed in and a new candidate for a prognostic indicator in prostate cancer. J Urol. 2004;**171**(1):187–91. PubMed PMID: 14665873.
8. Klezovitch O., Chevillet J., Mirosevich J., Roberts R.L., Matusik R.J., Vasioukhin V. Hepsin promotes prostate cancer progression and metastasis. Cancer Cell. 2004;**6**(2): 185–95. PubMed PMID: 15324701.
9. Kelly K.A., Setlur S.R., Ross R., Anbazhagan R., Waterman P., Rubin M.A., Weissleder R. Detection of early prostate cancer using a hepsin-targeted imaging agent. Cancer Res. 2008;**68**(7):2286–91. PubMed PMID: 18381435.