^{131}I-Labeled arginine-arginine-leucine (RRL)-containing cyclic peptide (YCGGRRLGGC) for imaging prostate carcinoma

^{131}I-Labeled RRL peptide

Liang Shan, PhD[1]

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Chemical name:	131I-labeled arginine-arginine-leucine (RRL)-containing cyclic peptide (YCGGRRLGGC) for imaging prostate carcinoma	
Abbreviated name:	^{131}I-Labeled RRL peptide	
Synonym:	WT1040	
Agent Category:	Peptide	
Target:	Integrin $\alpha_v\beta_3$	
Target Category:	Receptor	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{131}I	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

Integrins are transmembrane glycoproteins with two noncovalently bound α and β subunits. The two subunits mediate cell–cell and cell–extracellular matrix (ECM) interactions, and they act downstream of several primary signaling events that lead to

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

Corresponding author.

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angiogenesis (1-4). Integrins comprise a large family of cell adhesion molecules, and integrin $\alpha_v\beta_3$ appears to be the most attractive member for angiogenesis-targeted imaging and therapy because of its critical involvement in tumor angiogenesis, development, and metastasis. Integrin $\alpha_v\beta_3$ is minimally expressed in normal blood vessels but is significantly upregulated in newly sprouting vasculature in tumors (2, 4, 5).

A significant effort has been made to generate various imaging agents targeting integrin $\alpha_v\beta_3$ (1, 4, 6-9). Generally, these agents can be categorized as antibodies, peptides, small-molecule peptidomimetics, and targeted nanoparticles. Vitaxin is the representative of monoclonal antibodies against integrin $\alpha_v\beta_3$. Vitaxin is a humanized antibody composed of human IgG-1, kappa, and the complement domain regions of the murine antibody LM 609. Regardless of its efficacy in inhibiting angiogenesis, imaging with ^{99m}Tc-labeled Vitaxin failed to show the tumor angiogenesis in the clinical settings because of its poor stability and rapid plasma clearance with low doses (10). Interestingly, Gutheil et al. have reported that second-generation Vitaxin-2–conjugated, gadolinium-encapsulated nanoparticles could provide enhanced and detailed imaging of rabbit carcinomas and imaging of angiogenic "hot spots" that are not seen with standard magnetic resonance imaging (11). The major limitations of monoclonal antibodies include large molecular size, low production yield, incomplete tumor penetration, and immunogenicity to host.

Numerous small peptides have been identified to specifically interact with tumor neovasculature, including arginine-glycine-aspartic acid (RGD), asparagine-glycine-arginine, histidine-tryptophan-glycine-phenylalanine, and arginine-arginine-leucine (RRL) (7, 12-16). RGD tripeptide sequence is known as a cell recognition site for adhesive proteins present in the ECM and in blood. Integrin $\alpha_v\beta_3$ binds ECM proteins through the exposed RGD tripeptide in their ligands. Both linear and cyclic RGD peptide agents have been proven to be useful in imaging tumor neovasculature. In general, linear peptides are broken down rapidly *in vivo* and occupy a wide range of conformations, resulting in low binding affinity and less specific accumulation within tumors. Short cyclic peptides are superior to linear peptides for their pharmacokinetics due to the fact that they are trapped in the active conformation and are more resistant to proteolysis. The pharmacokinetic behaviors of RGD peptides can be further improved with introduction of sugar moiety, the dimeric format of RGD peptides, or coupling with 1,4,7,10-tetraazacyclododecane-*N,N',N",N"'*-tetraacetic acid or polyethylene glycol (12, 13, 15). However, RGD peptides are less selective (binding with 8 of the 24 integrins), and their binding affinities are relatively low (50% inhibition concentration (IC_{50}), 20–70 nmol) compared to antibodies. A series of non-peptide $\alpha_v\beta_3$ chemical antagonists including IntegriSense (IC_{50}, 4.1 nmol; binding with $\alpha_v\beta_3/\alpha_v\beta_5$ only) have recently been shown to accumulate specifically in $\alpha_v\beta_3/\alpha_v\beta_5$-expressing tumors with high affinity and specificity (14). More recently, several classes of peptidomimetic integrin $\alpha_v\beta_3$ antagonists have been reported (6, 17). These peptidomimetic antagonists consist of a rigid core scaffold bearing basic and acidic groups that mimic the guanidine and carboxylate groups of the RGD sequence. Additionally, ligand array of integrin antagonists on nanoparticles has been proven to be a viable strategy to target vascular surface receptors on endothelial cells. Imaging studies are in progress with these new strategies (6).

Nevertheless, small peptides have distinct advantages such as easy synthesis, less immunogenicity, and rapid plasma clearance. In addition to RGD, the tripeptide sequence RRL has been investigated to generate imaging agents (16, 18). RRL is a tumor endothelial cell–specific binding peptide previously identified by Brown et al. using an *in vitro* bacterial peptide display library panned against tumor cells (19). Weller et al. have shown that the microbubble-linked RRL peptide preferentially adheres to tumor *versus* normal vasculature, and this selective adherence can be detected with ultrasound (18). Yu et al. studied the radionuclide-labeled RRL peptide (designated WT1040) for tumor imaging and demonstrated that the ^{131}I-labeled RRL peptide allowed non-invasive visualization of tumors (16).

Related Resource Links:

- Chapters in MICAD
- Integrin-related articles in OMIM
- Integrin á$_V$â$_3$-related clinical trials in ClinicalTrials.gov

Synthesis

[PubMed]

The RRL peptide (Tyr-Cys-Gly-Gly-Arg-Arg-Leu-Gly-Gly-Cys-NH_2) and the control peptide (Tyr-Cys-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Cys-NH_2) were synthesized with the solid-phase peptide synthesis method, and the disulfide bonds between cysteines on each peptide were formed to maintain the cyclic structure. The cysteine at the C-terminal of RRL and control peptides was amidated to protect the peptides from rapid biodegradation. Radioiodination of the peptides was performed with the chloramine-T method (16). The best RRL peptide/chloramine-T weight ratio was 1:1.8. When the ratio differed from this value, the radiolabeling yield decreased. When the reaction time was 1, 3, 5, and 10 min, the radiolabeling yield reached 60, 62, 65, and 65%, respectively. When the input of peptide was 10, 30, and 50 µg, the radiolabeling yield reached 40.3, 49.7, and 60%, respectively. The radiochemical purity of ^{131}I-labeled RRL peptide was >95%. The molecular weight was 1040.2 before oxidation and 1038.2 after oxidation. The specific activity was not reported. After incubation at 37°C with human blood serum, the radiochemical purities of ^{131}I-labeled RRL peptide were 96.1 ± 0.40, 95.7 ± 0.35, 95.5 ± 1.38, 94.8 ± 0.35, and 90.3 ± 0.63% at 1, 2, 4, 8, and 24 h, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No references are currently available.

Animal Studies

Rodents

[PubMed]

Yu et al. analyzed the biodistribution of ^{131}I-labeled RRL and compared it with that of control peptide in 4–6-week-old male BALB/c nude mice bearing PC3 human prostate carcinoma xenografts (16). The tissue uptake was measured at 1, 6, and 24 h after injection (n = 3 mice/group). Highest uptake for both radiotracers was observed in the kidney, liver, and intestine. There was no difference for the tumor uptake between ^{131}I-labeled RRL and control peptides at 1 h after injection ($P > 0.05$), but the ^{131}I-labeled RRL signal decreased more slowly over time in tumors (74% from 6 to 24 h) than in the kidneys (86%), muscle (90%), and other organs. Radiolabeled RRL peptide accumulated in the tumor at ~2.52% and ~0.65% injected dose per gram of tissue (ID/g) at 6 h and 24 h after injection, respectively, higher than in other organs ($P < 0.05$), whereas this value for ^{131}I-labeled control peptide was only 0.73% and 0.06% ID/g (both $P < 0.05$) at 6 h and 24 h, respectively. The tumor/tissue ratios (with the exception of the lung) increased with time for RRL peptide but did not increase for the control peptide. At 1, 6, and 24 h after injection, the tumor/blood ratios were 0.32, 1.12, and 1.30 for RRL peptide and 0.30, 0.37, and 0.22 for control peptide, respectively, and the tumor/muscle ratios were 1.40, 3.94, and 9.08 for RRL peptide and 1.98, 2.89, and 1.78 for control peptide, respectively. No blocking studies were carried out.

Single-photon emission computed tomography of mice bearing human prostate carcinoma after injection of ^{131}I-labeled RRL peptide showed high radioactivity in the thyroid, abdomen, and bladder at 1 h and in the thyroid and abdomen at 3 h, and showed low radioactivity in the tumor at both 1 h and 3 h. At 24 h after injection, high radioactivity was observed in the tumor, and the surrounding background was very low. The thyroid was no longer detected (16).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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