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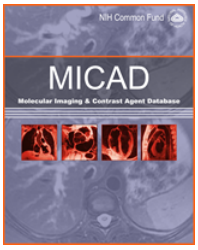

^{123}I-c(RGDfK)-human serum albumin-tissue inhibitor of matrix metalloproteinase 2 fusion protein

^{123}I-RGD-HSA-TIMP2

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Chemical name:	^{123}I-c(RGDfK)-human serum albumin-tissue inhibitor of matrix metalloproteinase 2 fusion	
Abbreviated name:	^{123}I-RGD-HSA-TIMP2	
Synonym:	^{123}I-RGDfK-HSA-TIMP2	
Agent category:	Protein	
Target:	$\alpha_v\beta_3$ integrin receptor	
Target category:	Receptor	
Method of detection:	Single-photon emission computed tomography (SPECT), gamma planar imaging	
Source of signal/contrast:	^{123}I	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently 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). 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 (3).

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Several families of MMPs are involved in atherogenesis, myocardial infarction, angiogenesis, and tumor invasion and metastases (4-7). MMP expression is highly regulated in normal cells, such as trophoblasts, osteoclasts, neutrophils, and macrophages. Elevated levels of MMPs have been found in tumors associated with a poor prognosis for cancer patients (8). There are four members of endogenous tissue inhibitors of metalloproteinases (TIMPs), which regulate the activity of MMPs leading to inhibition of tumor growth and metastasis (9, 10). TIMP-2 (TIMP2) is a bifunctional inhibitor of angiogenesis by inhibition of proteinase activity of MMPs and endothelial cell proliferation *via* binding to $\alpha_3\beta_1$ (the N-terminal domain) and by MMP-independent anti-angiogenic activity (the C-terminal domain) (11, 12). Kang et al. (13) fused the N-terminal domain of TIMP2 to the C-terminus of human serum albumin (HSA) to form HSA/TIMP2 fusion protein (HSA-TIMP2), which is readily secreted by the transfected yeast *Saccharomyces cerevisiae*. HSA-TIMP2 retains its anti-angiogenic activity at the C-terminal domain with little MMP inhibitory activity at the N-terminal domain. Lee et al. (14) have evaluated Cy5.5-HSA/TIMP2 (Cy5.5-HSA-TIMP2) for *in vivo* near-infrared (NIR) fluorescence imaging of rat prostate MLL tumors in nude mice showing maximum tumor accumulation at 2 d after injection.

Integrins are a family of heterodimeric glycoproteins on cell surfaces that mediate diverse biological events involving cell–cell and cell–matrix interactions (15). Integrins consist of an α and a β subunit and are important for cell adhesion and signal transduction. The $\alpha_v\beta_3$ integrin is the most prominent receptor affecting tumor growth, tumor invasiveness, metastasis, tumor-induced angiogenesis, inflammation, osteoporosis, and rheumatoid arthritis (16-21). Expression of the $\alpha_v\beta_3$ integrin is strong on tumor cells and activated endothelial cells, whereas expression is weak on resting endothelial cells and most normal tissues. The peptide sequence Arg-Gly-Asp (RGD) has been identified as a recognition motif used by extracellular matrix proteins (vitronectin, fibrinogen, laminin, and collagen) to bind to a variety of integrins, including $\alpha_v\beta_3$. The $\alpha_v\beta_3$ antagonists are being studied as anti-tumor and anti-angiogenic agents (18, 22, 23). Various radiolabeled RGD peptides (antagonists) have been introduced for imaging of tumors and tumor angiogenesis (24). Choi et al. (25) conjugated multiple c(RGDfK) peptides to HSA-TIMP2 to enhance the binding capacity of the protein (RGD-HSA-TIMP2) to tumors and their vasculatures. ^{123}I-RGD-HSA-TIMP2 and ^{123}I-HSA-TIMP2 have been studied as potential single-photon emission computed tomography (SPECT) probes for imaging $\alpha_v\beta_3$ receptors in nude mice bearing human glioblastoma U87MG tumors.

Related Resource Links:

- Chapters in MICAD (MMP, TIMP, RGD)
- Gene information in NCBI (TIMP2, HSA, α_v integrin, α_3 integrin, β_1 integrin, β_3 integrin)
- Articles in Online Mendelian Inheritance in Man (OMIM) (TIMP2, HSA, α_v integrin, α_3 integrin, β_1 integrin, β_3 integrin)
- Clinical trials (TIMP, RGD)
- Drug information in FDA (TIMP, RGD)

Synthesis

[PubMed]

HSA-TIMP2 (0.23 μmol) and c[RGDfK($COCH_2SH$)] (1.39 μmol) were incubated with *N*-succinimidyl iodoacetate (3.52 μmol) to form a thioether linkage between the RGD peptide and HSA-TIMP2 protein (25). RGD-HSA-TIMP2 was purified with column chromatography. The molecular weights of RGD-HSA-TIMP2 and HSA-TIMP2 were calculated to be 88.42 kDa and 92.78 kDa with mass spectroscopy, respectively. There were 6 RGD molecules per RGD-HSA-TIMP2. ^{123}I-RGD-HSA-TIMP2 and ^{123}I-HSA-TIMP2 were prepared with the Iodogen method by incubation of ~11 nmol protein with 185 MBq (5 mCi) [^{123}I]NaI for 20 min at room temperature. The radiochemical yields were 45%–50%. The specific activities of ^{123}I-RGD-HSA-TIMP2 and ^{123}I-HSA-TIMP2 were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Flow cytometry analysis showed that 45% and 91% of U89MG cells were positive after incubation with HSA-TIMP2-FITC and RGD-HSA-TIMP2-FITC for 24 h at 4°C (25), respectively. Confocal microscopy showed internalization of fluorescence activity inside the cells incubated with 1.6 nM RGD-HSA-TIMP2-FITC for 3 h at 37°C but not with HSA-TIMP2-FITC. No blocking studies were performed with unlabeled RGD-HSA-TIMP2.

In vitro cell viability of U87MG was assessed after incubation with various concentrations (0.11–3.2 nM) of RGD, HSA-TIMP2, and RGD-HSA-TIMP2 for 24 h at 37°C (25). The cell viability values with 3.2 nM RGD, HSA-TIMP2, and RGD-HSA-TIMP2 were 92.5 ± 4.0%, 73.5 ± 7.9% ($P < 0.05$ *versus* RGD), and 66.7 ± 0.6% ($P < 0.001$ *versus* RGD), respectively.

Animal Studies

Rodents

[PubMed]

Choi et al. (25) performed whole-body SPECT/CT imaging in nude mice (the number of mice was not reported) bearing U87MG tumors at 1 h and 4 h after intravenous injection of 18.5 MBq (0.5 mCi) ^{123}I-RGD-HSA-TIMP2 or ^{123}I-HSA-TIMP2. Little tumor accumulation of ^{123}I-HSA-TIMP2 was observed at 1 h and 4 h. The tumor accumulation of ^{123}I-RGD-HSA-TIMP2 was slightly higher than that of ^{123}I-HSA-TIMP2, with poor tumor contrast. High liver accumulation for both tracers was observed. Immunofluorescence staining of tumor tissue sections showed an increase in CD31 (endothelial cell biomarker) expression in the tumor tissue.

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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