



T84.66 Anti-CEA diabody-GSTSGSGKPGSGEGSTSG-*Renilla* luciferase

Db-18-Rluc8

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Chemical name:	T84.66 Anti-CEA diabody-GSTSGSGKPGSGEGSTSG- <i>Renilla</i> luciferase	
Abbreviated name:	Db-18-Rluc8	
Synonym:		
Agent category:	Antibody	
Target:	Carcinoembryonic antigen (CEA)	
Target category:	Antigen	
Method of detection:	Optical Imaging	
Source of signal/contrast:	Luciferin	
Activation:	Yes	
Studies:	<ul style="list-style-type: none"> <i>In vitro</i> Rodents 	No structure is currently available in PubChem .

Background

[PubMed]

Carcinoembryonic antigen (CEA) is a highly glycosylated protein with a molecular mass of 180 kDa that belongs to the immunoglobulin supergene family (1). CEA contains ~60% carbohydrate and a protein portion of 668 amino acids. As a common human tumor antigen, CEA is overexpressed on >80% of colorectal, pancreatic, breast, non-small cell lung, and medullary cancers, and on >70% of gastric, invasive cervical, endometrial, urinary bladder, and head/neck cancers (2). A variety of antibodies have been developed to target CEA. For example, T84.66 is a murine monoclonal antibody with a molecular mass of 150 kDa with exceptionally high affinity ($2.6 \times 10^{10} \text{ M}^{-1}$) for the $\alpha_3\beta_3$ epitope on the CEA (2, 3). T84.66 has been used to quantify CEA expression in immunohistological staining and in *in vivo* imaging. The T84.66 diabody is a recombinant fragment of the antibody that comprises two identical single chain variable (scFv) regions (4). Each scFv consists of a V_L domain of 107 amino acids, a peptide linker of 8 amino acids (GGGSGGGG), and a V_H domain of 112 amino acids. The two scFvs associate asymmetrically to form a stable diabody (scFv dimer) with a molecular

mass of 55 kDa. T84.66 diabody retains the high avidity of T84.66 *via* its bivalent binding. The small size of the diabody allows for rapid *in vivo* targeting in imaging and therapeutic applications.

Renilla luciferase (Rluc) is a 36-kDa enzyme protein extracted from a bioluminescent soft coral (sea pansy (*Renilla reniformis*)) (5). Rluc can catalyze emission of light from substrates, i.e., the oxidation of coelenterazine to coelenteramide generates a green fluorescence (535–550 nm) (6). Unlike the luciferase obtained from the firefly (Fluc), the Rluc oxidation process does not depend on adenosine-5'-triphosphate (7). Thus, Rluc is suitable for use as an *in vivo* bioluminescent tag, i.e., Rluc has been reported as a marker of gene expression in various cells and as a reporter gene or cell tag for *in vivo* imaging (5). Rluc8 is a variant of Rluc that contains amino acid substitution at the following eight locations: A55T, C124A, S130A, K136R, A143M, M185V, M253L, and S287L (5). These substitutions enhance the enzymatic activity stability of the molecule (>200 h) and increase the light emission as much as four-fold. The small size of Rluc makes it suitable for fusion with other proteins to generate specifically labeled proteins for imaging applications.

T84.66 Anti-CEA diabody-GSTSGSGKPGSGEGSTSG-*Renilla* luciferase (Db-18-Rluc8) is an optical agent used for imaging CEA (5). Db-18-Rluc8 contains three components: a T84.66 diabody for targeting CEA, an Rluc8 for catalyzing the coelenterazine substrate to emit light for optical detection, and a peptide linker of 18 amino acids to connect the diabody portion and the luciferase portion. The three components were assembled *via* protein fusion. Db-18-Rluc8 allows for localization of CEA-positive tumors in the living animal.

Synthesis

[PubMed]

Venisnik et al. briefly described the preparation of Db-18-Rluc8 (5). The gene encoding the T84.66 anti-CEA diabody was fused to the Rluc8 gene from phRL-CMV vector *via* slice overlap polymerase chain reaction (PCR). During this process, a linker of 18 amino acids (GSTSGSGKPGSGEGSTSG) was inserted between the diabody and the Rluc8. The obtained Db-18-Rluc8 gene was inserted into the bacterial expression plasmid pKKtac using *Hind*III and *Eco*RI sites, and a *pelB* leader sequence was included to facilitate bacterial expression and protein purification. The fused protein Db-18-Rluc8 was extracted from periplasmic secretion and purified with Ni-NTA chromatography. Db-18-Rluc8 protein also was obtained from expression in NS0 murine myeloma cells (5), which was transfected *via* electroporation with the Db-18-Rluc8 gene in a pFF12 expression vector.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The stability of the luminescence activity produced by Db-18-Rluc8 was examined in mouse serum. The half-life of the enzymatic activity was found to be >200 h with a Michaelis constant of 1.6 μ M for catalyzing coelenterazine. The bimolecular functionality of Db-18-Rluc8 was evaluated on the basis of its ability to bind CEA (the diabody portion) and its ability to emit light (the luciferase portion). Db-18-Rluc8 appeared to bind N-A3 protein, a recombinant CEA fragment, and to emit light by oxidizing coelenterazine. The binding to CEA was further examined with flow cytometry analysis of CEA-expressing human LS174T colon carcinoma cells. After incubation for 45 min, 83.8% LS174T cells were stained positive for Db-18-Rluc8, similar to the proportion stained with the anti-CEA antibody (T84.1 murine IgG₁).

Animal Studies

Rodents

[PubMed]

The specificity of Db-18-Rluc8 binding to tumor was examined *in vivo* (5). Athymic mice ($n = 7$) bearing 140–200 μg LS174T tumors (CEA-positive) were intravenously injected with 100 μl of Db-18-Rluc8 solution (17 μg of total protein, 9.1×10^8 relative light units (RLU)). At 2, 4, 6, 8, and 24 h after injection, optical images were acquired with the cooled charge-coupled device camera immediately after intravenous injection of coelenterazine (0.7 mg/kg). Optical signal became observable at 2 h and increased over time. The tumor signal to tissue background ratio (S/B) peaked at 6 h (6.0 ± 0.8). At 24 h, the target was still visible with S/B ratio of 2.8 ± 0.7 . Athymic mice ($n = 7$) bearing 140–200 mg C6 rat glioma xenografts (CEA-negative) were examined as a control. The S/B ratio in the C6 tumors was 1.0 ± 0.1 at all time points, which was significantly lower than that in the LS174T tumors. Two additional control experiments were performed, including assessment the uptake of luciferase or coelenterazine in tumors and tissues. No specific targeting was found in either case. The specificity and dynamics of binding were further examined *in vivo* with ^{124}I -labeled Db-18-Rluc8 (5). Athymic mice ($n = 4$) bearing LS174T tumors (120–140 mg) or C6 rat glioma xenografts (45–100 mg) were injected intravenously with 140–150 μCi ^{124}I -labeled Db-18-Rluc8 solution. Positron emission tomography (PET) was performed 4 or 21 h after injection. At 4 h, the S/B ratio in the LS174T tumor was 3.8 ± 0.8 compared with 1.2 ± 0.3 in the C6 tumors. At 21 h, the S/B ratio increased significantly to 18.0 ± 5.3 in the LS174T tumor but retained the same level in the C6 tumor (1.6 ± 0.3). After collecting the PET images at 21 h, the animals were euthanized and tissues were harvested for quantification of uptake. Accumulation of ^{124}I appeared to be significantly high in the LS174T tumor ($12.9 \pm 1.8\%$ ID/g) compared with that in the C6 tumor ($1.7 \pm 0.40\%$ ID/g) and that in the tissue ($<0.8\%$ ID/g).

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.

NIH Support

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References

1. Thomas P., Toth C.A., Saini K.S., Jessup J.M., Steele G. Jr. The structure, metabolism and function of the carcinoembryonic antigen gene family. *Biochim Biophys Acta*. 1990; **1032** (2-3):177–89. PubMed PMID: 2261493.
2. Blumenthal R.D. Technology evaluation: cT84.66, City of Hope. *Curr Opin Mol Ther*. 2004; **6** (1):90–5. PubMed PMID: 15011786.
3. Neumaier M., Shively L., Chen F.S., Gaida F.J., Ilgen C., Paxton R.J., Shively J.E., Riggs A.D. Cloning of the genes for T84.66, an antibody that has a high specificity and affinity for carcinoembryonic antigen, and expression of chimeric human/mouse T84.66 genes in myeloma and Chinese hamster ovary cells. *Cancer Res*. 1990; **50** (7):2128–34. PubMed PMID: 2107969.

4. Carmichael J.A., Power B.E., Garrett T.P., Yazaki P.J., Shively J.E., Raubischek A.A., Wu A.M., Hudson P.J. The crystal structure of an anti-CEA scFv diabody assembled from T84.66 scFvs in V(L)-to-V(H) orientation: implications for diabody flexibility. *J Mol Biol.* 2003; **326** (2):341–51. PubMed PMID: 12559905.
5. Venisnik K.M., Olafsen T., Loening A.M., Iyer M., Gambhir S.S., Wu A.M. Bifunctional antibody-Renilla luciferase fusion protein for in vivo optical detection of tumors. *Protein Eng Des Sel.* 2006; **19** (10):453–60. PubMed PMID: 16882674.
6. Loening A.M., Wu A.M., Gambhir S.S. Red-shifted Renilla reniformis luciferase variants for imaging in living subjects. *Nat Methods.* 2007; **4** (8):641–3. PubMed PMID: 17618292.
7. Loening A.M., Fenn T.D., Wu A.M., Gambhir S.S. Consensus guided mutagenesis of Renilla luciferase yields enhanced stability and light output. *Protein Eng Des Sel.* 2006; **19** (9):391–400. PubMed PMID: 16857694.