Avidin conjugated to tetramethyl-6-carboxyrhodamine-QSY®7

Av-TM-Q7

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Chemical name:	Avidin conjugated to tetramethyl-6-carboxyrhodamine-QSY®7	
Abbreviated name:	Av-TM-Q7	
Synonym:		
Agent Category:	Receptor ligand	
Target:	D-galactose receptor	
Target Category:	Receptor	
Method of detection:	Optical imaging: fluorescence	
Source of signal / contrast:	Tetramethyl-6-carboxyrhodamine	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	Click here for the protein and nucleotide sequence of *Gallus gallus* (chicken) avidin.

Background

[PubMed]

Early detection of cancer helps in the development of a proper treatment and monitoring regimen that may result in a suitable prognosis for the patient (1-3). Although invasive methods are often used for the detection of cancer, they have limitations because these procedures may detect the neoplasm only in a specific location and cannot determine whether the cancer has metastasized to other parts of the body. Also, the various imaging techniques and modalities available for the non-invasive detection of cancer have low sensitivity and/or resolution that are insufficient to detect and quantify microscopic

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tumors or a small cluster of cancerous cells (4). In addition, some of the contrast agents used for imaging purposes are known to have toxic side effects (5). An attractive alternative to radionuclides or contrast agents is the use of fluorescent optical imaging agents that can be used to visualize and manage or treat clinical pathology, including that of cancer (6-9). However, fluorescent dyes used for imaging can be toxic or nonspecific, and the signal generated by these agents may be masked by autofluorescence, leading to a low signal/background ratio (10). Also, the attenuation of the fluorescent signal in the tissue is such that only superficial tumors can be detected in humans. Investigators have proposed the use of targeted fluorescent probes as a solution to improve the signal/background ratio with the optical imaging agents, and they have shown that fluorescence dye conjugated to avidin can be used to detect submillimeter disseminated peritoneal tumors under *in vitro* and *in vivo* conditions only if direct access to the peritoneal tumor is possible (11, 12). In addition to binding to biotin, avidin (a 68-kD tetrameric glycoprotein) is also known to bind to the D-galactose receptor, which belongs to the lectin class of molecules (proteins that can bind to the carbohydrate groups of glycoproteins and glycolipids) and has been reported to be expressed on the surface of ovarian, gastric, colon, and pancreatic cancer tumor cells (12, 13). Because of its specificity to bind to cell-surface ligands, the use of avidin-therapeutic drug conjugates as a method to deliver and facilitate the uptake of anticancer drugs by cancer cells was also suggested to treat this ailment (14). The net positive charge on avidin facilitates its binding to lectins or similar molecules that are negatively charged at physiological pH (11). Lectin-bound avidin or its conjugates are rapidly internalized by the cell, and the unbound avidin and its conjugates are quickly cleared from circulation through the liver. Therefore, this phenomenon helps generate very high target signal/background ratios in small animals (11).

Ogawa et al. proposed the use of fluorophores that could be activated only after binding to the target to further improve the target/background ratios obtained with the optical imaging agents (15). These investigators developed a targeted and activatable fluorophore-quencher (FQ) probe that could be used for the optical imaging of tumors. In the native state the signal from the fluorophore is quenched by a quencher molecule. A fluorescence signal would be obtained from the fluorophore only after the FQ probe was bound to, internalized, and activated by the target cells. The mechanism of quenching and generating fluorescence from the FQ probe is discussed elsewhere (16). Ogawa et al. investigated the use of tetramethyl-6-carboxyrhodamine (TAMRA, fluorophore)-QSY®7 (quencher) pair conjugated to avidin (Av-TM-Q7) for the detection of cancer cells under *in vitro* conditions and also in mice bearing SHIN3 cell line (of human ovarian cancer origin; these cells have a surface expression of the D-galactose receptor) tumors (15).

Synthesis

[PubMed]

The synthesis of Av-TM-Q7 was described by Ogawa et al. (15). Briefly, avidin was incubated with TAMRA-hydroxysuccinimide (NHS) ester in 0.1 M sodium phosphate

buffer (Na_2HPO_4) (pH 8.5) for 30 min at room temperature. The Av-TM conjugate was purified on a Sephadex G50 column. The purified product was concentrated and added to a solution of QSY®7-NHS in dimethylsulfoxide. The mixture was incubated as described above. The final product, Av-TM-Q7, was purified on a Sephadex G25 column and stored at 4°C until required. The stability of Av-TM-Q7 under these storage conditions was not reported. Approximately three and five molecules of TAMRA and QSY®7, respectively, were reported to be conjugated to each molecule of avidin, as determined with a UV-visible spectroscopy system.

To generate a control conjugate for use in some studies, Av-TM-Q7 was incubated with disuccinimidyl suberate (DSS) in 0.1 M Na_2HPO_4 buffer (pH 8.5) for 1 h at room temperature. Incubation with the DSS generated a cross-linked avidin tetramer (Av-TM-Q7 (CL)) that does not degrade in the lysosome under acidic conditions. The purification and stability of Av-TM-Q7 (CL) was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The quenching ability of Av-TM-Q7 was determined by exposing the conjugate to 5% sodium dodecylsulfate (SDS), which is known to dissociate the avidin tetramer into its component monomers, in phosphate-buffered saline at room temperature for 10 min (15). For comparison, the Av-TM-Q7 (CL) conjugate was also treated with SDS under the same conditions. Measurement of the fluorescence signal from each native and denatured sample showed that only the dissociated sample of Av-TM-Q7 had a 40-fold increase in the signal intensity over background. Little change was noted in the signal obtained from Av-TM-Q7 (CL) with or without the SDS treatment.

To demonstrate activation of Av-TM-Q7 or the Av-TM-Q7 (CL) conjugates, SHIN3 cells were exposed to the respective conjugates at 37°C for 6 h to allow internalization and activation of the fluorophore in the lysosomes (15). Examination of the exposed cells under a fluorescence microscope showed that fluorescent dots were visible only in the lysosomes of cells exposed to Av-TM-Q7. No fluorescence signal was obtained from cells treated with the cross-linked conjugate. From these observations the investigators concluded that degradation of avidin in the lysosomes was responsible for generation of the fluorescent signal due to release of the quencher from the fluorophore in the Av-TM-Q7 conjugate.

Animal Studies

Rodents

[PubMed]

Peritoneal tumors were generated in nude mice by intraperitoneal injections (i.p.) of either SHIN3 cells or SHIN3 cells transfected with a plasmid (ZsGreen) that constitutively

expressed the green fluorescent protein (SHIN3/ZsGreen cells), and the tumors were allowed to grow for 3 weeks (15). Imaging was performed on the animals 3 h after an i.p. injection of the respective avidin conjugates. The number of animals injected per conjugate was not reported. Peritoneal tumors, including the submillimeter nodules, were visible only in mice treated with the Av-TM-Q7 conjugate. A considerably lower fluorescence signal was observed from tumors in mice treated with Av-TM-Q7 (CL).

To determine specificity of the Av-TM-Q7 and Av-TM-Q7 (CL) conjugates, mice with the SHIN3/ZsGreen cell tumors (the number of animals injected with each conjugate was not reported) were given an i.p. injection of either Av-TM-Q7 or Av-TM-Q7 (CL) (15). An almost complete overlap of the fluorescent signal from TAMRA and ZsGreen was reported in tumors of these animals. With results obtained from this study, it was concluded that Av-TM-Q7 could be used to visualize D-galactose receptor–positive tumors in the peritoneal cavity of mice.

On the basis of the information obtained from the various studies performed with Av-TM-Q7, the investigators concluded that this activatable fluorescence-quencher probe could be a promising agent to manage cancer with a "see and treat" strategy if an optical probe could be introduced into the peritoneum (15).

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.

Supplemental Information

[Disclaimers]

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