Single-chain anti-epidermal growth factor receptor antibody fragment conjugated to functionalized quantum dots

ScFvEGFR-QD

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Chemical name:	Single-chain anti-epidermal growth factor receptor antibody fragment conjugated to functionalized quantum dots	
Abbreviated name:	ScFvEGFR-QD	
Synonym:		
Agent Category:	Single-chain antibody fragment (ScFv)	
Target:	Epidermal growth factor receptor (EGFR)	
Target Category:	Receptor	
Method of detection:	Fluorescence imaging	
Source of signal / contrast:	Quantum dots (QD)	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

The epidermal growth factor receptor (EGFR) family is a group of transmembrane glycoproteins that mediate their activity through a tyrosine kinase (TK) and regulate several normal cell functions such as growth, differentiation, proliferation, and angiogenesis (1, 2). The structure of the receptor and its various ligands are discussed

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elsewhere (3). A mutated EGFR or its overexpression have been correlated to various malignancies and indicate a poor therapeutic outcome for the patient (4-7). Because of its established role in the initiation, development, and metastasis of many malignant tumors, EGFR was identified as an excellent target for the treatment of cancers (8). Mishani et al. and Krause and Baumann have described various labeled monoclonal antibodies (MAbs), directed toward the extracellular (EC) domain of the EGFR because of their high target specificity, and small-molecule drugs (inhibitors directed toward the intracellular TK) that are often used alone or in combination with other drugs for the diagnosis or treatment of cancer under preclinical or clinical conditions (4, 8). However, the use of MAbs for the treatment of cancer has limitations because the large size of the molecules and long blood circulation half-lives were observed to be an impediment for their distribution within a tumor. Also, due to MAb excretion primarily through the hepatic route, radioactivity from labeled MAbs that have been used to detect cancer tends to accumulate in the liver and masks the detection of any tumors in this organ (9). In addition, cancer patients treated with TK inhibitors often develop resistance to these small-molecule drugs (10). Moreover, in general, patients treated with an anti-cancer MAb or a small-molecule drug show only a modest response to the treatment (11).

In an effort to circumvent limitations observed in cancer treatment with the bulky MAbs (~160 kDa), investigators have engineered smaller (~25–30 kDa) single-chain antibody fragments (ScFv) that consist of the heavy and light variable chains of an antibody linked with a flexible peptide. The ScFv fragments are approximately five times smaller than the native MAbs, and they have been reported to have the same affinity and specificity for the target antigen as the parent MAbs (12). On the basis of the assumption that the ScFv fragments could show superior efficacy compared with the intact MAbs used for cancer therapy, investigators identified, expressed, and purified different ScFv fragments against the EGFR EC and characterized them under *in vitro* conditions (13, 14). Zhou et al. (14) also investigated the binding and uptake of liposomal nanoparticles (NP) bearing the ScFv fragments directed against the EGFR EC (ScFvEGFR) using tumor cells overexpressing the EGFR (for a description of the various types of NPs and their uses, click here (PubMed)). Results obtained from that study showed that the liposomal ScFvEGFR could easily bind to the EGFR, was internalized by the tumor cells, and showed no cytotoxicity.

Yang et al. conjugated a ScFvEGFR to functionalized quantum dots (QDs), which are fluorescent NPs, to obtain ScFvEGFR-QDs and investigated their binding and internalization by EGFR-expressing cancer cells (15). Using a fluorescent imaging technique, the investigators demonstrated that ScFvEGFR-QDs specifically bound to and were internalized by EGFR-expressing cancer cells. In addition, the use of ScFvEGFR-QDs as a molecular imaging agent was demonstrated in an orthotopic human pancreatic cancer mouse xenograft model.

Synthesis

[PubMed]

The synthesis of ScFvEGFR-QDs was described by Yang et al. (15). Briefly, QDs with an emission at 580 nm were synthesized and coated with amphiphilic copolymers as described elsewhere (16, 17). The QDs were then conjugated through the carboxyl group on the amphiphilic copolymer surface to nickel-nitriloacetic acid (Ni-NTA) through its amino residue. The product particles, Ni-NTA-QDs, were then mixed with the ScFvEGFR containing a six-histidine tag in a 20:1 ratio in phosphate-buffered saline (PBS). The mixture was incubated at 4°C overnight, and the final ScFvEGFR-QD conjugate was purified on a Nanosep 100K OMEGA filter column. The number of ScFvEGFR linked to each QD, and the storage conditions and stability of ScFvEGFR-QDs, were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The specificity and internalization of ScFvEGFR-QDs and non-targeted QDs were investigated using various human cancer cell lines (15). To determine specificity, the ScFvEGFR-QDs and non-targeted QDs were incubated respectively with MDA-MB-231(human breast cancer cell line that is high EGFR expressing), and MCF-7 (human breast cancer cell line with low EGFR expression) cells grown on slides for 1 h at 37°C. The cells were then washed with PBS, fixed with ice-cold acetone, and examined under a fluorescence microscope. Only cells exposed to ScFvEGFR-QDs showed a red fluorescence on the cell surface, and no signal was observed with cells exposed to the non-targeted QDs.

To investigate internalization of the ScFvEGFR-QDs, MDA-MB-231 (high EGFR expression) and MCF-7 (low EGFR expression) cells grown on slides were exposed to either ScFvEGFR-QDs or the non-targeted QDs for 2 h at 37°C (15). The cells were then washed with PBS and fixed with ice-cold acetone. The fixed cells were examined under a fluorescence confocal microscope, and a strong signal was observed inside only the MDA-MB-231 cells exposed to ScFvEGFR-QDs. A weak signal was seen with the MCF-7 cells under the same experimental conditions. No signal was obtained with cells exposed to the non-targeted QDs.

Animal Studies

Rodents

[PubMed]

The biodistribution of ScFvEGFR-QDs was compared with that of the non-targeted QDs in nude mice bearing human pancreatic cancer cell line (MIA PaCa-2) xenograft tumors (15). The number of animals used for the two QD types in the study was not reported. The animals were injected through the tail vein with either ScFvEGFR-QDs or non-targeted QDs. The mice were euthanized 5 h after injection, and the tumors, along with the normal tissue, were removed to be frozen in nitrogen. The frozen sections of the various tissues were obtained and observed under either a fluorescence or a confocal microscope.

Compared with the non-targeted QDs, only the ScFvEGFR-QDs were observed in the tumor tissue with fluorescence microscopy. Observation of the tumor tissue under the confocal microscope showed that the ScFvEGFR-QDs were internalized by the tissue. An examination of the normal tissue with fluorescence microscopy revealed that the non-targeted QDs were present primarily in the liver, followed by the spleen, and at low and very low levels in the lungs and kidneys, respectively. Low levels of ScFvEGFR-QDs were detected in the liver and spleen, and very low levels were detected in the lungs and kidneys. From these observations, the investigators concluded that ScFvEGFR-QDs could possibly be used as *in vivo* tumor imaging agents.

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

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