Single-chain anti-epidermal growth factor receptor antibody fragment conjugated to magnetic iron oxide nanoparticles

ScFvEGFR-IO

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Chemical name:	Single-chain anti-epidermal growth factor receptor antibody fragment conjugated to magnetic iron oxide nanoparticles	
Abbreviated name:	ScFvEGFR-IO	
Synonym:		
Agent Category:	Single-chain antibody fragment (ScFv)	
Target:	Epidermal growth factor receptor (EGFR)	
Target Category:	Receptor	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal / contrast:	Iron oxide (IO) nanoparticles	
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 or the surrounding area (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). The liposomal ScFvEGFR were shown to bind to and be internalized by tumor cells overexpressing the EGFR.

In another study, Yang et al. conjugated the ScFvEGFR fragment to magnetic iron oxide (IO) NPs to obtain ScFvEGFR-IOs and investigated their binding and internalization by EGFR-expressing cancer cells (15). For the structure, function, and preclinical use of the IO NPs, please click here (PubMed); for clinical evaluation of IO NP please click here (clinical trials). Using the magnetic resonance imaging (MRI) technique, the investigators demonstrated that ScFvEGFR-IOs specifically bound to and were internalized by EGFR-expressing cancer cells. In addition, the use of ScFvEGFR-IOs as a molecular imaging agent was demonstrated with MRI in an orthotopic human pancreatic cancer mouse xenograft model.

Synthesis

[PubMed]

The synthesis of ScFvEGFR-IOs was described by Yang et al. (15). The IO NPs were coated with amphiphilic copolymers and modified with short polyethylene glycol chains. The linking of ScFvEGFR to IO NPs was performed with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The core size of the conjugated IOs was 25.4 ± 4.2 nm as determined with transmission electron microscopy and hydrodynamic light-scattering measurements. The final ScFvEGFR-IO conjugate was purified on a Nanosep 100K OMEGA filter column. The storage conditions and stability of ScFvEGFR-IOs were not reported.

Using the same technique, IO NPs conjugated to the green fluorescence protein (GFP-IO) were prepared for use as controls while performing MRI under *in vitro* conditions (15). On the basis of a standard curve drawn with the fluorescence obtained from different amounts of the GFP alone, ~8–10 GFP molecules were reported to be conjugated to each IO NP. The investigators assumed that the number of ScFvEGFR fragments linked to each IO NP was similar to the number of GFP molecules bound to the IO NP (15).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Yang et al. determined the specificity of ScFvEGFR-IO binding by exposing MIA PaCa-2 (human pancreatic cancer origin cells that express high levels of EGFR) and HEK293 (human embryonic kidney epithelial cells used as controls have low EGFR expression) xenograft tumor cells to ScFvEGFR-IOs and non-targeted IOs (15). The cells were then stained with Prussian blue (an iron-specific stain) and examined under the microscope. Only the MIA PaCa-2 cells showed a high level of binding of the targeted IO NP, and a few HEK293 cells showed a low level of targeted IO NP binding. In contrast, only a few MIA PaCa-2 cells were reported to bind the non-targeted IO NP, similar to the HEK293 cells.

To determine internalization of the ScFvEGFR-IOs, cultured tumor cells were exposed to either ScFvEGFR-IOs or GFP-IOs for 3 h at 37^{o} C, harvested and washed with phosphate buffered saline (pH of buffer not reported), and embedded in 1% agarose to perform MRI using a fast-spin T_2-weighted echo imaging sequence. Several echo time data points were collected (15). A drop in the T_2 signal intensity from ~5.6 to ~2 was reported within 150 ms only in the MIA PaCa-2 cells that were exposed to the targeted IOs. Cells exposed to GFP-IO showed a decrease from ~5.6 to ~3.75 under the same conditions, indicating that a significantly higher amount of ScFvEGFR-IO was internalized by the MIA PaCa-2 cells.

Animal Studies

Rodents

[PubMed]

The biodistribution of ScFvEGFR-IOs was compared with that of the non-targeted IOs in nude mice bearing human pancreatic cancer cell line (MIA PaCa-2) xenograft tumors (15). The number of animals used for the two IO types in the study was not reported. The animals were injected through the tail vein with either ScFvEGFR-IOs or non-targeted IOs, and MRI was performed at different time points after the injection. Changes in the T_2-weighted MRI signal showed that ScFvEGFR-IO accumulated primarily in the xenograft tumors from 5 to 30 h after administration of the targeted IOs. No such change was reported in tumors of mice treated with the non-targeted IOs. Using the MRI signal generated by muscle tissue as the baseline, a 4.8-fold change in the MRI signal was observed between tumors exposed to ScFvEGFR-IO and those exposed to the non-targeted IOs under *in vivo* conditions. To confirm observations obtained with MRI, Prussian blue staining of frozen tumors tissue sections was performed 48 h after injection of the two NP types. Tumor sections from mice treated with ScFvEGFR-IO were stained with Prussian blue, and an examination of the sections under high magnification showed that the stain was localized primarily within the cells. No such staining was evident in frozen tumor sections of mice treated with the non-targeted IOs. Compared with the muscle tissue, the relative contrast intensities of the liver, spleen, kidneys, and brain were ~0.6, 0.5, ~0.02, and <0.01, respectively, at 28 h after injection of the non-targeted IOs. The relative contrast intensities for these organs were 0.5, ~0.35, ~0.02, and ~0.05, respectively, at 28 h after injection of the targeted IOs. With results obtained from various studies, the investigators concluded that the ScFvEGFR-conjugated IO NPs could be detected with MRI in EGFR-expressing tumors under *in vivo* conditions.

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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