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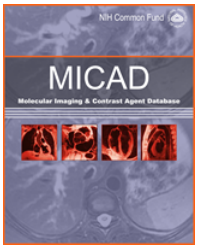

QD800-Anti-epidermal growth factor receptor monoclonal antibody nanoparticles

QD800-EGFR Ab

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Chemical name:	QD800-Anti-epidermal growth factor receptor monoclonal antibody nanoparticles	
Abbreviated name:	QD800-EGFR Ab	
Synonym:		
Agent category:	Antibody	
Target:	Epidermal growth factor receptor (EGFR)	
Target category:	Receptor	
Method of detection:	Optical, near-infrared (NIR) fluorescence	
Source of signal:	Quantum dot (QD)	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about HER2.

Background

[PubMed]

Epidermal growth factor (EGF) is a 53-amino-acid growth factor (6.2 kDa) secreted by ectodermic cells, monocytes, kidneys, and duodenal glands (1). EGF stimulates growth of epidermal and epithelial cells. EGF and at least seven other growth factors and their transmembrane receptor kinases play important roles in cell proliferation, survival, adhesion, migration, and differentiation. The EGF receptor (EGFR) family consists of four transmembrane receptors, including EGFR (HER1/erbB-1), HER2 (erbB-2/neu), HER3 (erbB-3), and HER4 (erbB-4) (2). HER1, HER3, and HER4 comprise three major functional domains: an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain. No ligand has been clearly identified for HER2. However, HER2 can be activated as a result of ligand binding to other HER receptors with the formation of receptor homodimers and/or heterodimers (3). HER1 as well as HER2 are

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overexpressed on many solid tumor cells such as breast, non-small-cell lung, head and neck, and colon cancer (4-6). The high levels of HER1 and HER2 expression on cancer cells are associated with a poor prognosis (7-10).

Optical fluorescence imaging is increasingly used to monitor biological functions of specific targets in small animals (11-13). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–600 nm) are used. Near-infrared (NIR) fluorescence (650–1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have a wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. NIR fluorophores also have high sensitivity, resulting from low fluorescence background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is a noninvasive alternative to radionuclide imaging in small animals (14, 15).

Fluorescent semiconductor quantum dots (QDs) are nanocrystals made of CdSe/CdTe-ZnS with radii of 1–10 nm (16, 17). They can be tuned to emit in a range of wavelengths by changing their sizes and composition, thus providing broad excitation profiles and high absorption coefficients. They have narrow and symmetric emission spectra with long, excited-state lifetimes, 20–50 ns, as compared with excited-state lifetimes of 1–10 ns for fluorescent dyes. They possess good quantum yields of 40%–90% and high extinction coefficients. They are more photo-stable than conventional organic dyes. They can be coated and capped with hydrophilic materials for additional conjugation with biomolecules, such as peptides, antibodies, nucleic acids, and small organic compounds, which were tested *in vitro* and *in vivo* (17-21). Although many cells have been labeled with QDs *in vitro* with little cytotoxicity, there are limited studies of long-term toxicity of QDs in small animals (22-30). However, little is known about the toxicity, and the mechanisms of clearance and metabolism of QDs in humans.

Trastuzumab is a humanized IgG_1 monoclonal antibody (Ab) against the extracellular domain of recombinant HER2, with an affinity constant (K_d) of 0.1 nM (31). ^{111}In-Trastuzumab, Cy5.5-trastuzumab, and ^{68}Ga-trastuzumab-$F(ab')_2$ have been developed for imaging of human breast cancer (32-35). Trastuzumab has also been successfully coupled with quantum dots for optical imaging of HER2 in tumors in mice (36). Yang et al. (37) conjugated EGFR monoclonal Ab to QDs with a maximum emission wavelength of 800 nm to produce QD800-EGFR Ab nanoparticles for use with *in vivo* imaging of U14 head and neck squamous cell carcinoma (HNSCC) in nude mice.

Related Resource Links:

- Chapters in MICAD (EGFR)
- Gene information in NCBI (EGFR)
- Articles in Online Mendelian Inheritance in Man (OMIM) (EGFR)
- Clinical trials (Trastuzumab)
- Drug information in FDA (Trastuzumab)

Synthesis

[PubMed]

A commercially available QD-antibody labeling kit was used to conjugate EGFR Ab with QDs to produce QD800-EGFR Ab nanoparticles (37). In brief, QDs were first activated with the heterobifunctional cross-linker 4-(maleimidomethyl)-1-cyclohexanecarboxylic acid *N*-hydroxysuccinimide ester (SMCC) to yield a maleimide-nanocrystal surface. Excess SMCC was removed with column chromatography. QD800-EGFR Ab was then reduced with dithiothreitol (DTT) to expose free sulfhydryl groups, and excess DTT was removed with column chromatography. The activated QDs were covalently coupled with reduced antibody for 60 min at room

temperature, and the reaction was quenched with ß-mercaptoethanol. QD800-EGFR Ab nanoparticles were purified with gel-filtration chromatography. The molar ratio of EGFR Ab to QD and the hydrodynamic diameter of QD800-EGFR Ab nanoparticles were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Yang et al. (37) performed cell-binding assays using confocal laser scanning microscopy analysis with QD800-EGFR Ab nanoparticles binding to human U14 cancer cells overexpressing EGFR. Incubation of 10 nmol (based on QD800) QD800-EGFR Ab nanoparticles for 30 min at 37°C showed high fluorescence intensity on the cellular membrane of U14 cells, whereas QD800 alone exhibited little fluorescence signal under the same incubation condition. Co-incubation with 1.32 pmol EGFR Ab inhibited the binding of QD800-EGFR Ab nanoparticles to the U14 cells.

Animal Studies

Rodents

[PubMed]

Yang et al. (37) used a fluorescence detection system after intravenous injection of QD800-EGFR Ab or QD800 nanoparticles (100 pmol QD/mouse) to study the tumor accumulation in nude mice (n = 5/group) bearing U14 tumors. Marked fluorescence intensity was observed with QD800-EGFR Ab in the tumors at 30 min and increased slightly with time up to 3 h after injection. Thereafter, the tumor fluorescence intensity decreased to a minimum by 24 h. The tumor/background ratios were 5.0 at 0.5 h, 5.4 at 3 h, 3.0 at 9 h, and 1.7 at 24 h. Pretreatment with excess EGFR Ab (1.65 nmol/mouse) decreased the fluorescence intensity to the background level to 0.5 at 24 h. Little fluorescence intensity was detected in the tumors at 0.5–24 h after injection of QD800. Confocal microscopy analysis of tumor section at 6 h and 24 h showed that QD fluorescence signal was not detected in the tumor sections from mice injected with QD800 or QD800-EGFR Ab plus EGFR Ab. In contrast, a strong fluorescence signal was detected in the tumor section from mice injected with QD800-EGFR Ab nanoparticles.

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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