Epidermal growth factor conjugated to near-infrared fluorescent quantum dots

EGF-QD

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Chemical name:	Epidermal growth factor conjugated to near-infrared fluorescent quantum dots
Abbreviated name:	EGF-QD
Synonym:	
Agent Category:	Receptor ligand
Target:	Epidermal growth factor receptor (EGFR)
Target Category:	Receptor
Method of detection:	Near-infrared fluorescence (NIR fluorescence)
Source of signal / contrast:	Quantum dots (QD)
Activation:	No
Studies:	• *In vitro* • Rodents

Structure of EGF quantum dot as shown by Diagaradjane et. al. (1)

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Background

[PubMed]

The epidermal growth factor (EGF) receptor (EGFR) is a membrane-spanning glycoprotein that mediates its activity through an intracellular tyrosine kinase (TK) signaling pathway and is considered to have a major role in the survival, growth, and proliferation of the mammalian cell (2). A mutated EGFR or its overexpression are thought to cause oncogenic transformation of cells that culminates in the development and progression of various types of cancers (2, 3). Because of its involvement in the cancer process, the EGFR and its associated TKs are targets of a variety of small-molecule and monoclonal antibody (MAb) inhibitors for the management and treatment of cancers (2, 3). However, these inhibitors are not entirely effective because patients either develop resistance to the small-molecule drugs or exhibit little to no response to the MAb treatment (4, 5). Several drugs and MAbs (and combinations of the two) are approved by the United States Food and Drug Administration for evaluation in clinical trials for the treatment of cancers. Some investigators in the field of anticancer drug development believe non-invasive imaging techniques used to visualize EGFR overexpression in tumors *versus* surrounding tissues could possibly predict the effectiveness of a therapeutic regimen directed against this ailment (1, 2, 6, 7). Although highly targeted radiolabeled MAb probes (~150 kDa) have been developed for the imaging of EGFR expression, these agents are not very useful because they have a large size and may not penetrate deeply into all tumors. Moreover, these molecules have long blood circulation half-lives, and the radiolabels tend to accumulate in organs involved in excretion of the radioactivity, thereby masking the metastatised tumors in these organs during imaging (8). The use of radiolabeled antibody fragments (~25 kDa) for EGFR imaging has also been attempted, but at present these agents appear to be primarily in preclinical development (9, 10).

As an alternative to radiolabeled probes, some investigators developed fluorescent dyes, which are easy and inexpensive to synthesize and do not expose a patient to radiation, and linked them to either anti-EGFR Abs or their fragments for the *in vivo* imaging of EGFR in animals (11, 12). However, these dyes show characteristic photobleaching, so the images must be acquired quickly; this is an imaging limitation because the fluorescence may not last for sufficiently prolonged periods. Therefore, it was necessary to develop an optical imaging agent with photo-stable characteristics to obtain high-quality images (1). Subsequently, fluorescent semiconductor quantum dots (QDs) were developed for molecular imaging and were shown to have superior imaging characteristics compared with the fluorescent dyes. The QDs have an emission spectra (20–30 nm) over a large

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excitation spectral range, have a longer fluorescence half-life (5–40 ns) compared with the dyes (0.5–2.0 ns), are resistant to photobleaching, and can be conjugated to a variety of biomolecules including peptides, nucleotides, etc (1, 7, 13, 14). Diagaradjane et al. conjugated EGF to near-infrared fluorescent QDs (EGF-QDs) and used them to image EGFR expression in human colon cancer cell lines under *in vitro* conditions and in xenograft tumors in mice (1). From these studies the investigators concluded that the EGF-QDs were a suitable imaging agent to determine the level of EGFR expression in cell lines and xenograft tumors.

Synthesis

[PubMed]

The synthesis of EGF-QDs was described by Diagaradjane et al. (1). Human recombinant EGF was obtained from commercial sources, and the QDs (emission maximum at 800 nm) were activated as detailed elsewhere (1). The activated QDs were purified on a Sephadex G-25 PD-10 desalting column with phosphate-buffered saline (PBS; pH 7.4) as the elution buffer. In a separate reaction, EGF was reduced with dithiothreitol for 30 min to obtain a product containing free sulfhydryl groups. The reduced EGF (r-EGF) was purified as described above for the activated QDs. The activated QDs and the r-EGF were mixed and allowed to conjugate for 1 h at room temperature. The reaction was quenched with β-mercaptoethanol, and the product was concentrated on a spin column (cut-off limit of spin column not provided). Purification of the EGF-QDs was performed with gel-filtration on a PD-10 column containing Sephadex G-200 with PBS (pH 7.4) as the elution buffer. The estimated concentrations of EGF and the QDs in the final product were determined to be 2.9 and 0.75 μmol/L, respectively, indicating there were four molecules of EGF conjugated to each QD (1). The storage conditions and stability of the EGF-QDs were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

With the use of an enzyme-linked immunosorbant assay, the native and r-EGF were reported to have a similar affinity for an anti-EGF Ab (1). However, Western blot analysis showed that, compared with the native EGF, the r-EGF induced a minimal phosphorylation of the EGFR under *in vitro* conditions. From these results the investigators assumed that the EGF-QDs would probably bind the EGFR on the cell surface, but would not trigger the EGFR signaling pathway in the cell, and the conjugated QDs would serve as good imaging probes.

Specificity of the EGF-QDs was determined by exposing human colorectal cancer HCT116 cells, which have a moderate expression of EGFR, and DiFi, which have high EGFR expression, to the conjugated QDs (1). Chinese hamster ovarian cancer K1 cells (CHO K1) that do not express the EGFR were used as controls for the study. Background fluorescence from the different cells was measured with confocal microscopy before the

cells were exposed to the EGF-QD conjugate for 20 min at 37°C. Two controls were set up for this study. As the first control, the different cell lines were respectively exposed to the native QDs alone. The second control involved pretreatment of the various cell lines with cetuximab, an anti-EGFR monoclonal antibody, for 45 min at 37°C followed by exposure to the EGF-QD nanoprobe. Fluorescent confocal microscopy showed that only the HCT116 and DiFi cells exposed to the EGF-QDs exhibited fluorescence within 5 min of treatment with the probe, and the signal correlated with the EGFR expression levels of these cells. No fluorescent signal was generated by the HCT116 and DiFi cells that were exposed to EGF-QDs after the cetuximab pretreatment or when exposed to the native QDs alone. From these studies the investigators concluded that the EGF-QDs bound specifically to the EGFR on these cells.

Animal Studies

Rodents

[PubMed]

In vivo imaging studies were performed with immunocompromised male nude mice bearing HCT116 xenograft tumors on the right flank (1). When the tumor size reached 0.8–1 cm in diameter, the animals were administered intravenously with either QDs alone (n = 7 animals) or the EGF-QD nanoprobe (n = 8 animals) through the tail vein. Near-infrared fluorescence imaging was performed on the animals at various time points starting at 3 min after treatment for up to 24 h. Among the various organs, the liver and the spleen showed the highest fluorescence with the QDs and with the EGF-QDs at 3 min after injection. This fluorescence decreased rapidly by 1 h after the injection, and only the EGF-QDs gave a high signal in these organs at 4 h post-injection (p.i.). By 24 h the QD and EGF-QD fluorescence reached baseline levels.

Using fluorescence obtained from the two groups of animals, the tumor/background ratios for the mice injected with the QDs or the EGF-QDs were determined at various time points (1). The QDs and the EGF-QDs showed a rapid influx at 3 min p.i. that gradually cleared by 60 min. The investigators reported that during this time the animals injected with EGF-QD exhibited a ~50% higher fluorescence compared with animals injected with the QDs alone. The clearance rate constants for the QDs and the EGF-QDs were 1.26 ± 0.38 per h and 0.57 ± 0.12 per h, respectively, during this period, and the half-lives of the QDs and the EGF-QDs were estimated to be 0.64 ± 0.15 h and 1.40 ± 0.32 h, respectively. By 24 h p.i. the fluorescence was reduced to background levels for both the QD and EGF-QD nanoprobes. The 4 h p.i. time point was reported to be the discriminating time point for the EGF-QD and QD fluorescence with the tumor/background ratios being 1.93 ± 0.27 and 0.86 ± 0.10, respectively ($P < 0.03$).

Pretreatment of the animals with cetuximab before the EGF-QD treatment abolished the tumor fluorescence observed in the mice that were not pretreated (1). From this result the

investigators concluded that the EGF-QDs had favorable pharmacokinetics and specificity for the EGFR under *in vivo* conditions.

For *ex vivo* microscopic fluorescence imaging, the various organs were extracted at 4 and 24 h p.i. from animals injected with either the QDs or the EGF-QDs (1). The liver and spleen showed the highest fluorescence with both probes at the two time points. The lungs had a higher intensity of fluorescence at 4 h than at 24 h. The kidneys had a higher fluorescence at 24 h than at 4 h with both probes. This was attributed to the possibly delayed renal clearance of the fluorescent probes from the animals.

A semiquantitative analysis of average fluorescence emitted from tissue homogenates was also performed at 4 and 24 h p.i (1). A significantly higher average fluorescence was reported in tissues from animals treated with EGF-QDs ($P = 0.0008$) compared with tissues obtained from the QD-treated animals. At 24 h p.i. no significant difference in average fluorescence ($P = 0.4137$) from the tissue homogenates of both animals groups was reported.

With results obtained from the various studies, the investigators concluded that the EGF-QDs had suitable pharmacokinetic, affinity, and specificity properties for use as an imaging tool to determine EGFR expression levels in xenograft tumors in mice (1).

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.

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