Cy5.5-Epidermal growth factor

Cy5.5-EGF

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Chemical name:	Cy5.5-Epidermal growth factor	
Abbreviated name:	Cy5.5-EGF	
Synonym:		
Agent category:	Polypeptide	
Target:	EGF receptor (EGFR, HER1)	
Target category:	Receptor	
Method of detection:	Optical, near-infrared (NIR) fluorescence	
Source of signal:	Cy5.5	
Activation:	Cy5.5-Epidermal growth factor	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about EGF.

Background

[PubMed]

Epidermal growth factor (EGF) is a 53-amino acid cytokine (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

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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 overexpressed on many solid tumor cells such as breast, non–small-cell lung, head and neck, and colon cancers (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 being used to monitor biological functions of specific targets (11-13). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-infrared (NIR) fluorescence (700–1,000 nm) detection avoids the natural background fluorescence interference of biomolecules, providing a high contrast between target and background tissues in small animals. NIR fluorophores have a wider dynamic range and minimal background fluorescence as a result of reduced scattering compared with visible fluorescence detection. NRI fluorophores also have high sensitivity, attributable to low background fluorescence, 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 non-invasive complement to radionuclide imaging in small animals.

Trastuzumab (a humanized immunoglobulin G_1 (IgG_1) monoclonal antibody against the extracellular domain of recombinant HER2) (14) and C225 (an anti-EGFR, chimeric, monoclonal antibody, also known as cetuximab) have been labeled as ^{111}In-trastuzumab (15-17) and ^{99m}Tc-EC-C225 (18, 19) for imaging EGFR expression on solid tumors using single-photon emission computed tomography (SPECT). However, antibodies that are approximately 25-fold larger than EGF may not be easily transported to cells within solid tumors. ^{111}In- and ^{68}Ga- labeled trastuzumab F(ab) antibody fragments have been used to partially overcome this permeability barrier (20, 21). Therefore, ^{111}In-EGF and ^{99m}Tc-HYNIC-EGF have been developed for SPECT imaging studies of tumors (22, 23). EGF has also been successfully coupled with Cy5.5 NIR dye for optical imaging of EGFR density in tumors in mice using non-radioactive materials.

Related Resource Links:

- Chapters in MICAD (EGFR)
- Gene information in NCBI (EGFR)
- Articles in OMIM (EGFR)
- Clinical trials (Trastuzumab, cetuximab)
- Drug information in FDA (Trastuzumab, cetuximab)

Synthesis

[[PubMed]

Commercially available Cy5.5 and monofunctional *N*-hydroxysuccinimide ester were used to conjugate EGF to form Cy5.5-EGF, which was purified by gel-filtration

chromatography (24). The molar ratio of Cy5.5 to EGF was estimated to be 0.5 as determined by spectrometric measurements. The yield of Cy5.5-EGF was 80–86%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Ke et al. (24) performed cell-binding assays with Cy5.5-EGF using human breast cancer cell lines MAD-MB-468 (EGFR-positive) and MDA-MB-435 (EGFR-negative). Incubation of Cy5.5-EGF (0.6 μM) for 1 h at 37°C resulted in high fluorescence intensity in the MAD-MB-468 cells, whereas no signal was observed in the MDA-MB-435 cells. On the other hand, Cy5.5 binding in both cell types was negligible. Binding of Cy5.5-EGF to MAD-MB-468 cells was completely blocked by C225 (4 μM) and EGF (10 μM).

Animal Studies

Rodents

[PubMed]

Ke et al. (24) studied the accumulation of Cy5.5-EGF in nude mice bearing MAD-MB-468 or MDA-MB-435 tumors using a whole-body fluorescence detection system. Cy5.5-EGF (2 nmol/mouse) was injected intravenously into nude mice bearing MAD-MB-468 tumors showing distinctly higher fluorescence signals as compared with MDA-MB-435 tumors at 24–48 h after injection. The intensity decreased to background levels by 192 h. Pretreatment with C225 inhibited the Cy5.5-EGF binding in the MAD-MB-468 tumors, whereas pretreatment with a nonspecific IgG antibody did not. The rate of accumulation of Cy5.5-EGF was significantly greater in the MAD-MB-468 tumors than in the normal tissue. There was no difference in the rate of Cy5.5-EGF accumulation between the MDA-MB-435 tumors and normal tissue.

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.

NIH Support

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