Ligand-conjugated, gold-doped CdHgTe quantum dots for multispectral imaging

QD800-RGD, QD820-anti-CEACAM1, and QD840-anti-EGFR

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Chemical name:	Ligand-conjugated gold-doped CdHgTe quantum dots for multispectral imaging	
Abbreviated name:	QD800-RGD, QD820-anti-CEACAM1, and QD840-anti-EGFR	
Synonym:		
Agent Category:	Nanoparticles	
Target:	Integrin αvβ3, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), and epidermal growth factor receptor (EGFR)	
Target Category:	Receptors	
Method of detection:	Optical imaging	
Source of signal / contrast:	Quantum dots (QDs)	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure available.

Background

[PubMed]

Three ligand-conjugated, gold-doped CdHgTe (Au:CdHgTe) quantum dots (QDs), abbreviated as QD800-RGD, QD820-anti-CEACAM1, and QD840-anti-EGFR, respectively, were synthesized by Han et al. for multispectral imaging (MSI) of multiple biomarkers in tumors (1).

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MSI is a technique that enables acquisition of spectrally resolved information at each pixel of an imaged scene. It solves some of the challenges in fluorescence-based imaging, e.g., eliminating tissue autofluorescence (2). In living subjects, MSI provides an effective means to quantify multiple biomarkers simultaneously (3, 4). To this end, QDs have been considered to be an attractive molecule label for use with MSI in the past decade because of their high fluorescence efficiency, minimal photobleaching, and constant excitation wavelengths together with sharp and symmetrical tunable emission spectra (5). QDs are spherical semiconductor nanocrystals, which are composed of elements from periodic groups of II–VI (CdSe) or III–V (InP) (5). They are typically synthesized by injection of liquid precursors into hot organic solvents, which enables generation of nanocrystals with different sizes by altering the amount of precursors and crystal growth time. The quantum yield reported in the literature ranges from less than 10% to up to 80%, depending on the materials, synthetic methods, and physicochemical properties of QDs (1, 6).

Han et al. synthesized an array of near-infrared (NIR) Au:CdHgTe QDs by doping gold into the CdHgTe QDs, which enhances the photoluminescence and reduces the cytotoxicity (1). The Au:CdHgTe QDs were monodispersed with tunable NIR fluorescence (740–840 nm). The quantum yield (QY) of Au:CdHgTe QDs reached 48%, which was higher than that of undoped CdHgTe QDs (42%) and Au nanoclusters (<2%) (1). To detect multiple tumor biomarkers simultaneously with MSI, these QDs were conjugated with three different ligands: RGD peptide targeting integrin $\alpha_v\beta_3$, and monoclonal antibodies targeting carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) and epidermal growth factor receptor (EGFR) (1). This chapter summarizes the data obtained with the three Au:CdHgTe QD conjugate mixtures.

Related Resource Links:

Nucleotide and protein information of integrin $\alpha_v\beta_3$

Nucleotide and protein information of CEACAM1

Nucleotide and protein information of EGFR

Synthesis

[PubMed]

Han et al. detailed the synthesis of the three Au:CdHgTe QD conjugates (1). Gold was first doped into the CdHgTe QDs by refluxing a mixed solution of $CdCl_2$, $HgCl_2$, $AuCl_3$, and KHTe for 5–60 min at 96°C under a slow N_2 flow in the presence of L-cysteine and L-glutathione as stabilizers. The Au dopant concentration (6.2% relative to the Cd molar concentration) was confirmed with inductively coupled plasma mass spectrometry. The emission peak shifted from 740 to 840 nm with prolonged refluxing time. The QY of as-prepared Au:CdHgTe QDs was 48% relative to that of 1,1'-dioctadecyltetramethyl indotricarbocyanine iodide (28%) under identical absorbance illumination conditions (0.037 a.u. at 747 nm).

The photostability of Au:CdHgTe QDs in water determined with spectrofluorometry was slightly higher than that of undoped CdHgTe QDs (1). After continuous optical excitation (λ_{ex}, 640 nm; λ_{em}, 840 nm) for 120 sec, the Au:CdHgTe QDs preserved 90.7% of their fluorescence, and the undoped CdHgTe QDs preserved 87.9%. Under transmission electron microscopy, the Au:CdHgTe QDs were uniform and monodispersed with an average diameter of 6–8 nm. The X-ray diffraction analysis of the powder Au:CdHgTe indicated that they had a cubic zinc blende structure, similar to the $CdHgTe_2$ (ICDD PDF 65-6126).

The RGD peptide and antibodies against CEACAM1 and EGFR were linked to Au:CdHgTe QD800, QD820, and QD840, respectively, with *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as cross-linkers. The QD/antibody and QD/RGD molar ratios were ~1:6 and ~1:160, respectively, in the conjugation reaction. The conjugation time was 1 h at room temperature. The excess quenching reagent and free RGD peptides were removed with ultrafiltration, and the QD-antibody conjugates were separated from free antibodies with gel filtration. The QD conjugates were verified with agarose gel electrophoresis. The conjugation efficiencies for RGD peptides and antibodies were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* cytotoxicity of Au:CdHgTe QDs was evaluated with A549 lung cancer cells after incubation with varied concentrations of QDs for 24 h (1). The half-maximal inhibitory concentration (IC_{50}) of the Au:CdHgTe QDs was measured to be 158.63 μg/mL (95% confidence limits: 142.79–182.60 μg/mL), whereas this value for the CdHgTe QDs was 84.17 μg/mL (95% confidence limits: 66.91–106.27 μg/mL). The A549 cells maintained >80% viability after treatment for 48 h with Au:CdHgTe QDs at concentrations as high as 100 μg/mL. In contrast, the viability of A549 cells treated with CdHgTe QDs at concentrations of 20–160 μg/mL decreased dramatically, suggesting that Au:CdHgTe QDs had a lower cytotoxicity than undoped CdHgTe QDs. Reduced cytotoxicity of the Au:CdHgTe QDs was considered to be due to the doping with Au and capping with glutathione and cysteine because the surface chemistry of QDs plays an important role in cytotoxicity in addition to the components of QDs themselves.

Animal Studies

Rodents

[PubMed]

The toxicity of QDs to mice was determined after tail vein injection of six doses (16, 20, 26, 32, 40, and 50 mg/kg body mass, respectively) of Au:CdHgTe QDs or CdHgTe to ICR mice (n = 5 males and 5 females/group) (1). The lethal dose (LD) measurement showed that the LD_5, LD_{50}, and LD_{95} of the Au:CdHgTe QDs were 25.35, 34.92, and 48.11 mg/kg,

respectively. The 95% confidence limits for LD_{50} of the Au:CdHgTe QDs were 31.37–39 mg/kg. The LD_5, LD_{50}, and LD_{95} for CdHgTe QDs were 19.11, 29.93, and 46.88 mg/kg, respectively, with 95% confidence limits of 26.34–33.98 mg/kg for LD_{50}. These results demonstrated that Au:CdHgTe QDs was less toxic to mice than CdHgTe QDs.

In vivo MSI was performed at 2 h after tail vein injection of the three QD conjugate mixture (~25 nM for each conjugate) to A549 tumor xenograft-bearing mice (1). In the mouse given QD conjugate mixture, the fluorescence signal was observed in the primary tumor, metastatic lesion, and lymphatic basin. The signals from QD800-RGD, QD820-anti-CEACAM1, and QD840-anti-EGFR were co-localized with certain differences in their distribution. Semi-quantification of the signal intensity (counts/sec) showed an order of lymphatic basin (0.41) > primary tumor (0.35) > metastatic lesion (0.17) for QD800-RGD; lymphatic basin (0.27) > metastatic lesion (0.16) > primary tumor (0.07) for QD820-anti-CEACAM1; and lymphatic basin (0.35) > metastatic lesion (0.25) > primary tumor (0.22) for QD840-anti-EGFR. The signal in the primary tumor from QD820-anti-CEACAM1 was much lower than that from QD800-RGD and QD840-anti-EGFR, which might be due to the expression difference of the three targets. These results indicated that the Au:CdHgTe QD conjugates could be used as probes for *in vivo* detection of multiple tumor markers simultaneously. The control mouse and mouse given unconjugated QDs showed no fluorescence signal in tumors.

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.

References

1. Han S., Mu Y., Zhu Q., Gao Y., Li Z., Jin Q., Jin W. *Au:CdHgTe quantum dots for in vivo tumor-targeted multispectral fluorescence imaging.* Anal Bioanal Chem. 2012;403(5): 1343–52. PubMed PMID: 22447216.
2. Byers R.J., Hitchman E.R. *Quantum dots brighten biological imaging.* Prog Histochem Cytochem. 2011;45(4):201–37. PubMed PMID: 21196026.

3. Cassette, E., Helle, M., Bezdetnaya, L., Marchal, F., Dubertret, B., and Pons, T. *Design of new quantum dot materials for deep tissue infrared imaging.* Adv Drug Deliv Rev. 2012
4. Zhou L., El-Deiry W.S. *Multispectral fluorescence imaging.* J Nucl Med. 2009;50(10): 1563–6. PubMed PMID: 19759119.
5. Tholouli E., Sweeney E., Barrow E., Clay V., Hoyland J.A., Byers R.J. *Quantum dots light up pathology.* J Pathol. 2008;216(3):275–85. PubMed PMID: 18814189.
6. Li J., Wu D., Miao Z., Zhang Y. *Preparation of quantum dot bioconjugates and their applications in bio-imaging.* Curr Pharm Biotechnol. 2010;11(6):662–71. PubMed PMID: 20497111.