

Bio-imaging of colorectal cancer models using a CK19-bioconjugated Quantum Dot Nanoprobe

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ABSTRACT

We describe the development of quantum dot (QD) probes for cancer targeting and imaging in living animals. Cadmium selenide (CdSe) QDs with a maximum emission wavelength of 655 nm, shelled with ZnS and a polymer coating presenting carboxylic groups were coated with streptavidin and functionalized with the biotinylated CK19-antibody. *In vivo* targeting studies of human colorectal cancer growing in mice indicated that the QD probes accumulate at tumors by antibody binding to cancer-specific cell surface biomarkers. Such approach promises to be highly desirable for molecular targeted research of gastrointestinal cancer.

Keywords: nanoparticle, nanoprobe, quantum dots, colorectal cancer, molecular imaging

1. INTRODUCTION

Visual analysis of biomolecules is an integral avenue of basic and applied biological research. Quantum dots (QDs) are nanocrystals with unique photophysical properties, including broad excitation spectra, size tunable

fluorescence, multi-color fluorescence with one single wavelength light source, high photostability and long fluorescence lifetime [1]. Molecular targeting/imaging is an important field in cancer research. Traditionally, organic dyes have been widely used as fluorescent labeling materials in a variety of applications including diagnostics and biological imaging. Nevertheless, there are serious limitations of organic fluorophores for biological applications, including narrow excitation bands and broad emission bands with red spectral tails, making simultaneous evaluation of several light-emitting probes problematic due to spectral overlap. Additionally, the resistance to photobleaching of many organic dyes was poor. Fluorescence *in vivo* imaging with quantum dot (QDs) probes, promises to greatly expand the capabilities of existing imaging modalities, providing access to high-resolution multiplexed vascular imaging, intraoperative image guidance, real-time cell tracking, and *in vivo* molecular targeting [1]. The aim of the study was to develop and validate a QD-nanoprobe for the detection of CK19 protein for *in vivo* colorectal cancer molecular targeting. We used CK19 since it is one of the main cytoskeleton proteins of epithelial cells and is released as

full-length protein from viable tumor cells and it is significant for the micrometastases detection [2]. This approach will further be used for the successfully separate cancer tissue from normal tissue helping surgeon's ability to delineate the tumor margins, therapy monitoring, *in vivo* sentinel lymph node mapping and micrometastases detection.

2. MATERIAL AND METHODS

In vitro binding affinity of these nanoprobe and unconjugated QDs was evaluated in a panel of cell lines and clinical samples [3]. Colorectal cancer cell line DLD-1 was used in this study. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂ in RPMI medium supplemented with 5% fetal bovine serum and 1% penicillin-streptomycin. For *in vivo* experiments, male NOD/SCID (NOD.CB17 Prkdcscid) mice (Charles River Laboratories International Inc., Wilmington, MA, USA), 6–8 weeks old, were used. Animals were injected s.c. with DLD-1 colorectal cancer cell at the two rear axillas (1×10^6 cells/100µl sterile PBS/injection). Tumors were measured twice per week using a digital caliper. The formula $(a \times b^2)/2$, where a = length and b = width, was used for the calculation of tumor size [4]. Upon reaching a tumor size of approximately 0.8 to 1 cm in diameter, the animals were administered an into the tail vein injection of 10 pmol QD nanoparticles (n = 3) and CK19-QD nanoprobe (n = 3) and imaged at various time points. *In vivo* optical imaging was done using the IVIS imaging system 50 series (Xenogen Corporation). CK19-QD showed CK19-specific binding *in vitro*. Animals were euthanized after the injection of QD and CK19-QD (at 3 h).

3. RESULTS

The potency, binding affinity and specificity of the synthesized CK19-QD nanoprobe were

shown using a quantum-dot-labelled magnetic immunoassay method [3]. CK19-QD bioconjugates were injected into control and tumor-bearing mice via vein tails for both tumor-targeted imaging. To evaluate the targeting efficiency of CK19-QDs, non-targeted QDs without CK19 were injected into tumor-bearing mice for comparison purpose. At the 3h time point, CK19-QD nanoprobe showed more prominent fluorescence when compared with QD nanoparticles (Figure 1).

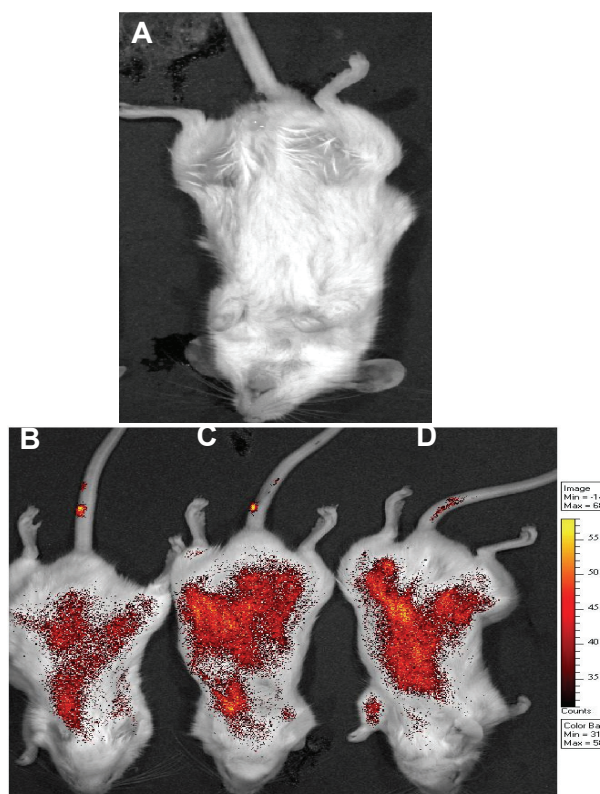


Figure 1. *In vivo* imaging results obtained from CK19-QD probes injected into the tail vein of control mouse (no tumor) (A) and tumor-bearing mouse (B-D). The site of QD injection was observed as a spot on the mouse tail.

The spectrum analysis showed that the fluorescence of tumor site was consistent with the distribution of CK19-QD probes, while the tissue adjacent to the tumor site showed no

characteristic peak of the QDs. The CK19-QD probes had broad excitation spectra and high fluorescence intensity. They could specifically and efficiently recognize CK19 antigen in colorectal cancer cells. CK19-QD probes have good optical properties and biocompatibility for *in vivo* targeted imaging of colorectal cancer.

4. DISCUSSION

We have developed a specific CK19-QD probe for colorectal cancer targeted imaging *in vitro* and *in vivo*, which has unique optical advantages, such as broad excitation bands, strong resistance to photobleaching and high fluorescence intensity. Under our usage dose, these probes have no obvious cytotoxicity to DLD-1 cells cultured *in vitro*.

For *in vivo* imaging, the CK19-QD probes could target the colorectal cancer specifically in the animal model without any significant acute toxicity. Our preliminary results suggested that our CK19-QD probes could be used as a safe and specific agent for colorectal cancer imaging. Thus, the surgeons were able to mark the tumor margins and further to remove them confidently with the help of nanoprobe. Such approach promises to be highly desirable for molecular targeted research of gastrointestinal cancer. At present, however, little is known about the mechanism of metabolism or clearance of QD probes injected into living animals. So, the possible mechanisms need to be examined carefully before semiconductor QDs are used in humans. We believe that QDs might be integrated with targeting, imaging and therapeutic agents to develop 'smart' nanostructures for noninvasive imaging, diagnosis and treatment of cancer.

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