Aptamer-conjugated dendrimer-modified quantum dots for glioblastoma cells imaging

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Abstract. Targeted quantum dots have shown potential as a platform for development of cancer imaging. Aptamers have recently been demonstrated as ideal candidates for molecular targeting applications. In present work, polyamidoamine dendrimers were used to modify surface of quantum dots and improve their solubility in water solution. Then, dendrimer-modified quantum dots were conjugated with DNA aptamer, GBI-10, can recognize the extracellular matrix protein tenascin-C on the surface of human glioblastoma cells. The dendrimer-modified quantum dots exhibit water-soluble, high quantum yield, and good biocompatibility. Aptamer-conjugated quantum dots can specifically target U251 human glioblastoma cells. High-performance aptamer-conjugated dendrimers modified quantum dot-based nanoprobes have great potential in application such as cancer imaging.

1. Development of Apt-dQDs nanoprobes

In recent years, molecular imaging of tumors has become a hotspot. Most probes for molecular imaging conjugate a targeting molecule to a reporter moiety. This study was aimed at developing a novel type of molecular imaging probes composed of aptamers (Apts), quantum dots (QDs), and polyamidoamine (PAMAM) dendrimers, for targeting to tumor cells.

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A previous SELEX experiment targeting U251 glioblastoma cells identified GBI-10 Apts that binds to tenascin-C, an extracellular matrix protein playing a important role in cancer cells proliferation and migration [1-2]. Therefore, we selected the GBI-10 as the targeting molecules for glioblastoma cells targeting and imaging.

QDs have been widely studied due to their unique optical properties, and have become a novel functional platform in bio-analytical science and molecular imaging [3-5]. However, some reports showed that QDs exhibit cellular toxicity [6]. How to decrease their toxicity and enhance their biocompatibility is a great challengeable problem. Dendrimers are a class of polymers with highly ordered structure. Our previous results demonstrate that the dendrimers functionalized nanomaterials such as CNTs, QDs and magnetic nanoparticles, markedly enhance the biocompatibility and cellular uptake of nanoparticles [7-9].

Herein, we used QDs and PAMAM dendrimers as raw materials, and fabricated dendrimer-modified QDs, then we selected tenascin-C over expressed in glioblastoma cells as research target, and conjugated dQDs with Apt, the resultant Apt-dQDs nanoprobes were used to investigate the feasibility of targeting and imaging U251 glioblastoma cells.

To obtain Apt-dQDs nanoprobes, we firstly prepared partially thiolated PAMAM dendrimers (see Figure 1), then we used ligand replacement method to obtain partially thiolated PAMAM dendrimers-modified QDs, then, surface amine groups on the surface of dendrimers were converted to carboxylic acids by reacting with excess glutaric anhydride, and then 5'-amino modified Apt were conjugated with dendrimer-modified QDs, the resultant Apt-dQDs nanoprobes were characterized by HR-TEM (see Figure 2a) and photoluminescence spectra(see Figure 2b). Results demonstrated that as-prepared nanoprobes displayed good dispersibility and water-solubility, and have strong fluorescent signals.

To determine if Apt were successfully conjugated to PAMAM dendrimers, we used agarose (2%) gel electrophoresis to separate the components in the conjugations since agarose gel electrophoresis can separate ssDNA Apt because of its secondary double strands structure(see Figure 3). The shift results demonstrate Apt-dQDs nanoprobes are unable to run on the gel, indicating Apt could be successfully conjugated with PAMAM dendrimers modified nanoparticles. Moreover, we could not observe the other bands in Apt-dQDs probes, suggesting that the byproducts and unconjugated Apt were removed successfully.

PAMAM G4.0, \( ^1H \) NMR (300 MHz, \( d^6\)-DMSO)
\[ \delta = 7.96 \text{ (br s, 60H), 3.08 \text{ (br s, 324H), 2.68 -2.42 \text{ (br s, 160H), 2.20 \text{ (br s, 64H) }} } \]
PAMAM-SH, $^1$H NMR (300 MHz, $d^6$-DMSO)

$\delta$ = 8.15 (br s, 63H), 3.74 (br s, 6H), 3.66 (br s, 12H), 3.16 (br s, 312H), 2.63-2.42 (br s, 160H), 2.20(br s, 58H)

Fig.1 The $^1$H NMR spectra of the G4.0 PAMAM dendrime and partially thiol-terminated G4.0 dendrimers.

Fig.2 Characterization of Apt-dQDs nanoprobes. (a) HR-TEM images of Apt-dQDs nanoprobes. (b) Photoluminscence spectra of QDs (solid line) and Apt-dQDs (dash line).

Fig.3 Gel electrophoresis results of Apt-dQDs nanoprobes after staining with ethidium bromide. Lanes 1, 2, 3, 4, and 5 represent the 100 bp DNA ladder, Apt, Apt-dQDs nanoprobes, dendrimer, and dQDs alone, respectively.

2. The cell imaging

To evaluate the targeting of Apt-dQDs nanoprobes, we firstly measured the binding ability of as-prepared nanoprobes to U251 glioblastoma cells. As shown in Fig.4a, U251 glioblastoma cells incubated with Apt-QDs nanoprobes, exhibit strong red color, and could not be washed
away, which suggest that the Apt-QDs nanoprobes can bind with U251 glioblastoma cells with high binding affinity. Conversely, control Scb10-dQDs conjugation can not bind with U251 glioblastoma cells (see Figure 4b). Therefore, we considered that Apt-QDs nanoprobes can target U251 glioblastoma cells specifically.

3. Conclusions
We have developed a class of Apt-conjugated nanoprobes, which could specifically bind to cancer cells and exhibits in vitro molecular imaging. These nanoprobes have great potential in applications such as in vitro and in vivo glioblastoma molecular imaging in near future.

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