Quantum dot-A10 RNA aptamer-doxorubicin conjugate

QD-Apt(Dox)

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Chemical name:	Quantum dot-A10 RNA aptamer-doxorubicin conjugate	
Abbreviated name:	QD-Apt(Dox)	
Synonym:		
Agent category:	Nucleic acid (nanoparticle)	
Target:	Prostate-specific membrane antigen (PSMA)	
Target category:	Nucleic acid binding protein	
Method of detection:	Optical imaging	
Source of signal/contrast:	Quantum dots, doxorubicin	
Activation:	No	
Studies:	• *In vitro*	No structure is currently available in PubChem.

Background

[PubMed]

Prostate-specific membrane antigen (PSMA) is a type II membrane glycoprotein with a molecular weight of ~100 kDa (1). PSMA is composed of several domains, including a potential phosphorylation site in the cytoplasmic tail (amino acids 1–18), a highly hydrophobic α-helix in the transmembrane region (amino acids 19–43), and catalytic sites in the extensive extracellular domain (amino acids 44–750). Two unique enzymatic functions are found in PSMA: *N*-acetylated, α-linked, dipeptidase (NAALADase) activity and folate hydrolase activity. As a prostate cancer cell marker, PSMA expression is primarily prostate-specific, with very low levels (~1,000-fold less) in the brain, salivary

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glands, and small intestine. PSMA has become an excellent target for imaging and therapy prostate cancer.

Aptamers (from the Latin *aptus*, to fit, and the Greek *meros*, part or region) are single-stranded or double-stranded oligonucleotides (RNA or DNA, respectively) that are modified to bind a variety of targets with high binding affinity and specificity (2). Aptamers range in size from 20 to 80 base pairs (~6–26 kDa) with dissociation constants in the range of 10 pM to 10 nM (3). Unlike linear oligonucleotides, which contain genetic information or antisense oligonucleotides that interrupt the transcription of genetic information, aptamers are globular molecules with a shape similar to tRNA and bind to target proteins specifically (4). A10 RNA aptamer (Apt) is a nuclease-stabilized 2'-fluoropyrimidine RNA molecule of 57 base pairs with a molecular weight of 18.5 kDa (5). Its 2'-fluoro-modified ribose on all pyrimidines and 3'-inverted deoxythymidine cap provide significant resistance to nuclease in blood (6). Apt has a single 5'-CG-3' sequence in its predicted double-stranded stem region, which is a preferred binding site for the anthracycline class of anticancer drugs such as doxorubicin (Dox) (7). Dox intercalates within the GC pair in Apt to form physical conjugate Apt(Dox) at molar ratio of 1.11:1 (dissociation constant = 600 nM) and emit fluorescence simultaneously. Because Dox possesses high efficacy against a range of neoplasms, including acute lymphoblastic and myeloblastic leukemias, malignant lymphomas, soft tissue and bone sarcomas, and breast, ovarian, prostate bladder, gastric, and bronchogenic carcinomas (8), this complex can be used as a PSMA-specific drug carrier to deliver Dox to prostate cancer cells.

Quantum dots (QDs) are semiconductor nanocrystals 2 to 10 nm in diameter (200–10,000 atoms) that possess a quantum confinement effect (hence the name "quantum dots") caused by the restriction of electrons and holes in all three dimensions (9, 10). Like classic semiconductors that are composed of two types of atoms from the II/VI or III/V group elements in the periodic table, these nanocrystals have a valence band and a conduction band separated by an energy gap (band gap). Upon excitation, an electron is promoted from the filled valence band to the largely empty conduction band, which creates a positive vacancy "hole" in the valence band. The spatial separation (Bohr radius) of this electron-hole pair ("exciton") is typically 1–10 nm for most semiconductors (10). The quantum confinement arises when one of the dimensions in the nanocrystals becomes comparable to its Bohr radius, at which time these valence/conduction bands are quantized with an energy value that is directly related to the nanocrystal size. Thus, the excitons are confined in a manner similar to a particle-in-the-box problem, leading to a finite band gap and discretization of energy levels. When the electron fills the vacancy in the valence band, light of a certain wavelength is emitted, which corresponds to the respective band gap energy that is a function of nanocrystal size. For instance, the emission wavelength is 550 nm for 3-nm CdSe QDs and 650 nm for 7-nm CdSe QDs (11, 12). For biological applications, QDs are generally encapsulated with biocompatible polymers, functionalized for various bioconjugations, and widely used to label molecules for optical imaging.

The QD-Apt(Dox) conjugate is an PSMA-specific agent used for optical imaging of the delivery of the anticancer drug Dox (13). QD-Apt(Dox) consists of three components: PSMA-specific Apt covalently attached to the core surface as targeting molecule and drug carrier, an anthracycline class of anticancer drug Dox as a therapeutic agent and optical sensor, and a carboxyl core-CdSe/ZnS shell QD (QD490) as a carrier of A10 and Dox and as the second optical sensor. The two sensors (Dox and QD) generate an optical signal *via* formation of bi-molecular fluorescence resonance energy transfer (Bi-FRET) complex. FRET is a near-field dipole-dipole interaction that involves energy transfer between two molecules in close proximity (3-6 nm). In QD-Apt(Dox), there is a donor-acceptor relationship between QDs and Dox. The fluorescence of both QD and Dox is quenched ("OFF") when QD-Apt is loaded with Dox, and fluorescence is restored upon the release of Dox ("ON") after the uptake of QD-Apt(Dox) by prostate cancer cells. Thus, QD-Apt(Dox) can be used to image drug delivery to prostate cancer cells.

Synthesis

[PubMed]

Bagalkot et al. reported the detailed preparation of QD-Apt(Dox) (13). Initially, commercially available carboxyl core-CdSe/ZnS shell QD was reacted with *N*-hydroxysuccinimide (NHS) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The produced QD-NHS was then covalently linked to 5'-NH_2-modified Apt at a molar ratio of 1:10 to yield QD-Apt conjugate. The excess active esterson the QD surface were quenched with ethanolamine 1 h later. Finally, QD-Apt was incubated with Dox to form QD-Apt(Dox).

The mechanism of Dox binding to QD-Apt was examined by fluorescence spectroscopy (13). For a fixed concentration of QD-Apt (10 μM) incubated with an increasing molar ratio of Dox (0 to 8), a sequential decrease in the fluorescence emission spectrum of QD (440–560 nm) was observed when excited at 350 nm. The maximal fluorescence occurred at the molar ratio of 1:7. This result suggested a diminished QD fluorescence caused by an energy transfer from QD to Dox. On the other hand, for a fixed concentration of Dox (10 μM) incubated with an increasing molar ratio of QD-Apt (0 to 0.16), a sequential decrease was also found in the fluorescence emission spectrum of Dox (520–640 nm) when excited at 480 nm, confirming that the quenching of Dox fluorescence was caused by intercalation within Apt on the surface of QDs. These experiments demonstrated that the formation of the QD-Apt(Dox) produced a Bi-FRET system, in which the QD fluorescence was quenched by Dox through a donor-acceptor model between QD and Dox.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Bagalkot et al. studied the specificity of QD-Apt binding to PSMA with *in vitro* fluorescent microscopy (13). QD-Apt (100 nM) was incubated with PSMA-expressing prostate cancer cells (LNCaP) and PSMA-negative PC3 cells for 0.5 h at 37°C. A significant uptake of QD-

Apt was found in the LNCaP cells compared with that in the PC3 cells. On the basis of these results, QD-Apt(Dox) was assumed to bind specifically to LNCaP cells. The uptake of QD-Apt(Dox) by LNCaP cells was examined with confocal scanning microscopy as a function of incubation time (13). After incubation for 0.5 h, a faint signal was observed for both internalized QD and Dox (an "OFF" state of Bi-FRET system). At this time, the majority of Dox remained intercalated within the surface Apt, leading to a quenching of both QD and Dox fluorescence. After incubation for 1.5 h, a substantial increase was observed in both QD and Dox fluorescence (an "ON" state of Bi-FRET system). At this time, a substantial amount of Dox was released from the QD-Apt(Dox) caused by either the physical dissociation of Dox from QD-Apt(Dox) or biodegradation of the surface Apt in the lysosomes. Both QD and Dox generated very sharp images of LNCaP cells with low background noise, which allowed *in vitro* detection of cancer cells at the single-cell level. The cytotoxicity of QD-Apt(Dox) was assessed with an *in vitro* methylthiazole tetrazolium (MTT) cell proliferation assay (13). LNCaP cells appeared to have lower cellular viability (52.5 ± 1.6%, $n = 3$) than PC3 cells (77.2 ± 3.1%, $n = 3$). Because free Dox, QD, and Apt exhibited no apparent cytotoxicity to LNCaP cells or PC3 cells, the higher cytotoxicity of QD-Apt(Dox) found in LNCaP cells might be related to the release of Dox molecules after endocytotic uptake.

Animal Studies

Rodents

[PubMed]

No publication is currently available.

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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