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DNA Walker-Regulated Cancer Cell Growth Inhibition

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Abstract

We demonstrate a DNAzyme-based walker system as a controlled oligonucleotide drug AS1411 release platform for breast cancer treatment. AS1411 strands are released from fuel strands as a walker moves along its carbon nanotube track. The release rate and amount of anti-cancer oligonucleotides are controlled by the walker operation. With the walker system embedded within the collagen extracellular matrix, we show that this drug release system can be used for in-situ cancer cell growth inhibition.

Graphical Abstract

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DNA walkers that release anti-cancer drugs: A DNAzyme walker system is designed as an in-situ drug release platform for cancer cell treatment. The walkers are embedded in the collagen extracellular matrix where breast cancer cells are cultured, and release anti-cancer oligonucleotide AS1411, regulating cancer cell growth.

Keywords

DNA walker; DNAzyme; anti-cancer oligonucleotides; cancer cell growth inhibition

DNA walkers, inspired by intracellular protein motors, are a class of synthetic molecular motors that can move processively along prescribed pathways.^[1] Typically, a single-stranded DNA or multiple strands form a walker system with a series of anchor strands on a track template.^[2] A free energy gradient is created within the system such that the walker moves spontaneously toward a lower free energy configuration. Such biochemical reaction may be initiated by addition of fuel strands^[3] or activity of DNAszymes and restriction enzymes^[4]. The walking cycle or single turnover reaction can be completed by DNA strand displacement, returning to the initial higher free energy configuration.^[5] Depending on the walking mechanisms and system designs, it is possible to demonstrate autonomous translocation and/or directional control.

While a variety of DNA walker mechanisms have been studied, research on their applications has been limited. A major effort toward this goal has been the modification of DNA strands to incorporate functional molecules or nanoparticles as their payloads, thus to create DNA walker mediated transport or reaction systems. He and Liu constructed a DNA walker mediated organic synthesis system by exploiting the step-by-step walking process.^[6] Seeman and co-workers designed a molecular assembly unit where gold nanoparticles are picked up and transported to desired designations.^[7] We previously demonstrated an autonomous DNAszyme-based walker that transports fluorescent nanocrystal quantum dots (QDs) along carbon nanotubes (CNTs).^[8] We showed that the transport kinetics and processivity of the walker can be controlled by DNAszyme sequence and structure as well as metal cation type and concentration in buffer.^[9]

In the present study, we explore this CNT-based DNAszyme walker system as a controlled drug release platform for breast cancer treatment. The anti-cancer drug AS1411 oligonucleotides are released from fuel strands as the DNA walker moves along the CNT track where the fuel strands are decorated. The DNAszyme catalytically cleaves the prearranged part of the fuel strands, releasing AS1411 that inhibits the growth of human breast cancer cells (MCF-7). We show that the cleavage rate and the amount of released AS1411 can be controlled by regulating walker kinetics, ultimately determining the cancer cell proliferation. Finally, as a proof-of-principle, we demonstrate this DNA walker system as an in-situ drug regulating platform by embedding the walkers within the collagen extracellular matrix (ECM) where MCF-7 cells are cultured. The walker operation renders the growth rate of breast cancer cells to be only 60% of those of untreated MCF-7 cells. This DNA walker based drug release system is potentially advantageous over polymer or nanoparticle based drug delivery methods, as the drug release profiles could be precisely controlled by regulating the walker operation.^[10]

Figure 1 shows the principle of the DNA walker mediated in-situ drug release platform embedded in the collagen ECM. In this work, 10–23 DNAszyme (green), the 23rd clone after the 10th round of in-vitro selection, is used as the walker strand. It has a catalytic core and two flanking recognition arms.^[11] Initially, the CdTe/CdS QD (orange dot) bound DNAszyme is associated with a fuel strand which has two parts – upper AS1411 drug (light blue) and lower anchor strand (navy) – on the CNT track (see Supporting Information for details). The DNAszyme hydrolyzes the rArU site (brown dot) between the AS1411 and the

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anchor strand through metal cation mediated deprotonation.^[12] As the cleaved AS1411 diffuses away, the upper arm of the DNAzyme migrates to the next available fuel strand along the track. Finally, the lower arm displaces from the anchorage and migrates to the fuel strand, completing the so-called single turnover reaction. Continuous repetition of the single turnover events propels the autonomous processive translocation of the walker. The AS1411 segment (5'-GGT GGT GGT GGT TGT GGT GGT GGT GGrA -3') released from the DNAzyme and anchor strands forms a dimeric guanine (G) quadruplex. AS1411 is one of the G-rich oligonucleotides (GROs), which is an anti-proliferative drug toward multiple cancer cell lines at relative low concentrations, while it does not show fatal effects to normal cells at the same concentration level.^[12] The oligonucleotide drug is currently in phase II clinical trials. By incorporating the DNA walkers in the ECM, we demonstrate that the cancer cell growth can be regulated in situ by the drug released from the walker system.

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The DNA motor walking was first measured at the single molecule level using single-particle/single-tube spectroscopy.^[8] While the DNAzymes transport the QD along the immobilized CNT track, the QD and CNT are used as visible and near-infrared fluorophores, given their emission properties in their respective spectral range. In our custom-built inverted microscope, the visible QD fluorescence was measured with an electron-multiplying charge-coupled device (EMCCD), while the near-infrared CNT emission was recorded with a 2D InGaAs photodiode array. The localized QD fluorescence is monitored as the position (red dot) of the DNAzyme over a time period against stationary CNT image (Figure 2a). The QD position is recorded every two hours to allow the walker to travel sufficiently long distance. The single motor imaging was performed in standard TAE buffer containing 10 mM Mg²⁺, which is needed for catalytic cleavage of rArU by the DNAzyme. The DNA walker travelled approximately 700 nm after 6 hours, displaying a speed of ~110 nm/hr or single turnover reaction rate of $8.9 \times 10^{-3} \text{ s}^{-1}$ with an assumption that the average distance between two neighboring fuel strands along the CNT track is ~3.5 nm.^[9]

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The walker speed or single turnover reaction rate is confirmed by an ensemble measurement. The CNT-based DNA walkers were deposited on an agarose gel in a microfluidic channel (Figure 2b), where the released AS1411 strands decorated with Cy5 dye (red dot in Figure 1b) were collected at the outlet at a flowrate of ~67 $\mu\text{L/hr}$ for 3 hours. It should be noted that this low flowrate does not affect the walker operation significantly,^[8] and the translocation kinetics are dominated by the walker mechanism depicted in Figure 1b.^[9] From the collected solution, Cy5 fluorescence was measured and the amount of released AS1411 strands was determined. With knowledge of the number of AS1411 strands and the time period, the single turnover reaction rate is calculated to be $9.2 \times 10^{-3} \text{ s}^{-1}$, which reasonably agrees with the single motor measurement (Figure 2c).

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The release of AS1411 is a function of walker kinetics, which strongly depends upon the environmental factors such as Mg²⁺ concentration. Thus, the Cy5 fluorescence was measured to estimate the amount of AS1411 release as a function of Mg²⁺ concentration (Figure 2d). Approximately twice greater amount of AS1411 was released at 10 mM Mg²⁺ than at 5 mM, while no significant AS1411 release was observed at 0 mM as expected. The release of AS1411 in the cell culture medium (Advanced DMEM/F12) was also examined.

The medium contains roughly 1 mM Mg^{2+} , so additional magnesium ions were added for the final concentration of 10 mM. At this Mg^{2+} level, the Cy5 emission indicates that the release of AS1411 in the culture medium is similar to that in TAE buffer. These results suggest that DNAzyme walkers may be used as an AS1411 release platform that can function in cell culture environments.

AS1411 forms a dimeric G-quadruplex structure (Figure 1b) in the presence of 50 mM K^+ . This secondary conformation was probed by circular dichroism spectroscopy from 220 to 320 nm. The difference in left- and right-handed circularly polarized light absorption provides evidence of such oligonucleotide conformation. The peak at ~265 nm and the valley at ~240 nm in Figure 2e suggest that the released AS1411 indeed forms the G-quartet conformation, similar to the control as-received AS1411 strands. The cell culture medium contains 5 mM potassium ions, and thus, AS1411 is expected to retain its secondary structure which helps prevent the sequence from being degraded by nuclease activities, thus retaining its anti-cancer properties.^[13]

We then examined the efficacy of the released AS1411 in anti-proliferation of MCF-7 cells. Here, we performed the viability of MCF-7 cells (Figure 3a). Initially, approximately 60 cells were placed in each well of a 96-well plate. After 24-hour culture, the cells were exposed to various concentrations of AS1411; anti-cancer strands were added to the cell culture medium at the final concentration of 0.5, 5, and 20 μM . Here, the AS1411 strands released from the walker system were collected from the microchannel and examined along with as-purchased AS1411. We collected AS1411 strands up to 5 μM for drug treatment, due to the limited concentration of initial carbon nanotube samples. After 96-hour incubation, Hoechst dye was used to stain cells for fluorescence imaging to evaluate their viability. The blue spots in Figure 3c represent the cells stained by Hoechst, while the dead cells are washed away.^[14]

It is clearly seen in Figure 3a that there is statistically no difference in the drug efficacy for growth inhibition at 0.5 μM of AS1411 compared to the control cells (i.e. no AS1411 added). At 5 μM , however, the cell viability is approximately 50% of the control, demonstrating the efficacy of the anti-cancer oligonucleotides. Even greater efficacy is observed at 20 μM with approximately 20% viability. It should be noted that the released AS1411 shows nearly the identical level of cytotoxicity as the as-purchased AS1411 samples (strands which did not go through the release process), verifying that the walker system does not affect the function of AS1411 and that the additional base (rA) does not affect its efficacy (also see Figure S3). AS1411 strands target and bind to nucleolin, a protein overexpressed on the surface of cancer cells.^[15] The AS1411-nucleolin binding may affect cancer cell growth via nucleolin-mediated destabilization of mRNA for B-cell lymphoma 2 (BCL-2) proteins, ultimately resulting in the anti-proliferation of the cells.^[16]

To examine the target specificity, normal fibroblast CCD-1064SK cells were also exposed to the same level of AS1411 dosage. At 5 and 20 μM , there is no cytotoxicity at these high concentrations compared with control cells with no AS1411 added (Figure 3b). It should be also noted that CNT-based walkers were not co-cultured with cells in this work. While several reports in the literature demonstrated no adverse effects of carbon nanotubes in

cellular and small animal studies, there are a number of studies that raised concerns about their toxicity.^[17] Since the cytotoxicity of carbon nanotubes is beyond the scope of this study, we did not attempt to interface nanotube tracks with any cells directly.

With the anti-cancer effects of AS1411 on MCF-7 cells in conventional 2-dimensional (2-D) cell culture environment on 96-well plates, the walker system was explored in collagen ECM, which is closer to in-vivo tumor microenvironment. Here, the walkers were added to the collagen during the polymerization process such that the walkers are embedded within the ECM. Then, the MCF-7 cells were cultured on the top of collagen ECM, where they were exposed to diffusion of AS1411 strands released by the walker operation, while with no direct contact with the CNT-based walkers. As a control, MCF-7 cells were also cultured on a collagen ECM containing only CNT tracks decorated with fuel strands (i.e. AS1411), but no walker strands. Thus, no AS1411 strands were expected to be released from the CNT tracks. Figure 3c shows the bright-field image (top-row) and fluorescence image (bottom-row) of Hoechst stained cells on the collagen ECM. These images indicate that the less number of MCF-7 cells were populated on the DNA-walker-embedded collagen ECM than the control cells on the ECM with no walker system after 5-day incubation. As the statistical result is shown in Figure 3d, the viability of MCF-7 cells exposed to the released AS1411 is significantly lower at 60% compared to that of control cells. In contrast, there is no statistical difference in the viability between the control cells and the cells cultured on the ECM with AS1411-decorated CNT tracks alone (i.e. without walkers), as anticipated. With knowledge of the amounts of CNTs and associated fuel strands incorporated within the collagen ECM, we estimate a total amount of AS1411 strands to be roughly 5 μ M. At this concentration, the cell viability is slightly higher than that of the 2-D culture experiment at 5 μ M AS1411 in Figure 3a. Here, the drug treatment conditions are different; for the 96-well plate cell culture, the culture medium containing 5 μ M AS1411 was refreshed every 24 hours 4 consecutive days. In contrast, a maximum of 5 μ M AS1411 strands were released from the collagen ECM and the cell viability was evaluated after 5 days. Therefore, the actual drug amount and exposure time result in the difference in the cell viability and anti-proliferation behaviors. While the 2-D cell culture in the 96-well plate confirmed the anti-cancer properties of released AS1411 strands as designed, the ECM cell culture was performed to demonstrate in-situ cancer cell treatment via walker-regulated drug release.

In summary, a DNA-walker-mediated platform that inhibits cancer cell growth in situ is created as a proof-of-concept in this work. AS1411 oligonucleotides are used as a model anti-cancer drug. Given the robustness of the release platform, our walker system may be extended for treatment of other cancer cell lines with appropriate drugs.^[18] Finally, we envision that the highly precise reaction pathways of the walker system could be exploited for additional regulating mechanisms in response to, for example, external photo-irradiation or environmental changes by sequence designs with functional moieties, forming a unique powerful molecular regulating system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) DNA walker mediated platform for inhibiting cancer cell growth. MCF-7 breast cancer cells are cultured on the collagen ECM, where the DNA walkers are embedded. (b) Walking mechanism. As the DNAzyme (green) conjugated with a CdTe/CdS QD (orange) moves along the CNT track (black), anti-cancer oligonucleotide AS1411 (light blue) is released from the anchor strand (navy). The released AS1411, labelled with Cy5 dye (red), forms a dimeric G-quadruplex structure, necessary for anti-proliferation of MCF-7 cells.

Figure 2.

(a) The translocation of the DNAzyme walker is visualized by visible/near-infrared single-particle/single-tube spectroscopy. The CdTe/CdS QD and CNT track are used as visible and near-infrared fluorophores, respectively. The position of the QD (red dot), indicating the DNAzyme walker, was monitored against the immobilized CNT track over 6-hour period in standard TAE buffer containing 10 mM Mg^{2+} . The red arrow represents the walker trajectory. The speed of the walker is approximately 110 nm/hr. (b) Schematic of a microfluidic channel where an ensemble of DNA walkers operated on an agarose film in TAE buffer with 10 mM Mg^{2+} at room temperature. The AS1411 strands released by walker operation were collected at the outlet. (c) Single turnover reaction rates from the ensemble and single motor measurements from (a) and (b). (d) PL spectra of Cy5-labelled AS1411 strands collected as a function of Mg^{2+} concentration in 1×TAE buffer (blue, red, black). The AS1411 collected in cell culture medium is also presented in pink. (e) Circular dichroism spectra of released and as-received AS1411 strands in the presence of 10 mM K^+ .

Figure 3.

(a) Viability of MCF-7 breast cancer cells after drug treatment for 96 hours. Both released and as-received AS1411 strands are compared with control cells. (b) Viability of CCD-1064SK normal fibroblast cells after 96-hour incubation with AS1411. (c) Brightfield (top row) and fluorescence (bottom row) images of MCF-7 cells on collagen ECM after 120-hour culture. (d) Viability of MCF-7 cells cultured on the collagen ECM after 120-hour

AS1411 treatment. NS: not significant; *: p-value < 0.05. The cell viability is determined as the ratio of the area covered by AS1411-treated cells to the area covered by control cells.

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