PHARMACIA

Pharmacia 69(2): 285–293
DOI 10.3897/pharmacia.69.e80595

Review Article

Theranostic nanomachines for cancer treatment

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Received 16 January 2022 ♦ **Accepted** 7 February 2022 ♦ **Published** 5 April 2022

Citation: Loukanov A, Kuribara A, Filipov C, Nikolova S (2022) Theranostic nanomachines for cancer treatment. Pharmacia 69(2): 285–293. https://doi.org/10.3897/pharmacia.69.e80595

Abstract

Multifunctional programmed nanomachines with theranostic functions demonstrated great potential in the clinical practice of oncology, as well as the personalized nanomedicine. The reason is because such nanoagents with combined diagnostic and therapeutic functions were found to be highly effective for cancer treatment. The appropriate design of nanomachines allows them to overcome the biological barriers of proliferative tumors and to distinguish the cancer cells from their normal counterparts. Moreover, the use of biocompatible and biodegradable precursors for construction of nanomachines minimize significantly the caused adverse effects to the normal tissue cells, which is a main problem of the chemotherapy. In addition, the utilization of theranostic nanomachines also enables an improved selectivity to the cancer in respect to its intrinsic complexity, heterogeneity, and dynamic evolution. Here we present the programmable functions and performance of the microenvironment-responsive nanomachines at a molecular level for cancer imaging and therapy.

Keywords

Multiparameter theranostic nanomachines, nanoscale mechanical devices, nanoscale manipulation

Introduction

The progress of nanomedicine emerges the transition of conventional to personalized treatments and it is expected to have a great impact on the precision health care in near future (Fornaguear and Garcia-Celma 2017). The rapid development of theranostic nanomachines offers tremendous potential for precise medication with a proper dose in the right time and in a manner far superior to the traditional therapeutics (Loukanov et al. 2019). The integration of imaging and therapeutic functions into a single nanoscale smart device is emerging as a paradigm towards personalized nanomedicine because it allows precise diagnosis and individualized selection of treatment modality (Li et al. 2014). The nanomachine's design ensures imaging functions (i.e. fluorescence, magnetic resonance imaging, photoacoustics or computed tomography) with therapeutic components (i.e. chemotherapy, photodynamic therapy, or photothermal therapy), which enable simultaneous advanced diagnostic and therapeutic interventions in the patients (Loukanov et al. 2020). To successfully achieve its multifunctionality, the nanomachine must be biocompatible and programmed with aim to be enough "intelligent" to overcome all *in vivo* biological barriers during its mission in the body. This is of particular importance in the case of malignant cancers because of their intrinsic complexity, dynamic evolution, as well as intratumor heterogeneity in abnormal microenvironment (Sun and Yu 2015). Nevertheless,

the abnormal expression of biomarker proteins on the cancer cell membranes is an excellent target for specific recognition. Therefore, the nanomachine design must be developed in a programmable fashion that provides regulation of its properties and behavior for implementing of targeting, sensing, imaging, and therapy (Loukanov et al. 2018). One of the grand advantages of the nanomachine is the capability for targeting and/or attaching to the diseased cells based on bio-analysis of their multiple surface biomarkers or antigens, as well as soluble cues in their vicinity (Peng et al. 2020). But how such fascinating properties have been created? There are numerous of man-made monoclonal antibodies designed for application in the targeted therapy (e.g. cancer chemotherapy), but their usage might have limited numbers of treatments and potential to cause multiple accompanied side effects, such as high blood pressure, serious kidney damage, bleeding, blood clots etc. (Majidi et al. 2009). On the other hand, the functional oligonucleotides (also known as "chemical antibodies") or unnatural peptides (Minchev and Sofroniev 1987; Timcheva et al. 1987; Petkov et al. 1989; Sofroniev et al. 1989) have been successfully demonstrated as alternative of those protein antibodies, because of their ability to select and bind the target cells (Sun et al. 2014). For example, screened aptamers may serve as antagonists of the natural ligands to activate, enhance, or block the bioactivity of overexpressed receptors. They can be also used to deliver cytotoxic drugs or other disease-related therapeutic agents to target cells as cancer or tumor tissue (Xuan et al. 2018). The oligonucleotide functions are far beyond the conventional genetic roles of the nucleic acids. Another reason for it is that DNA fibers are great materials for design and fabrication of nanomechanical devices (Seeman 2005). It is because of their highly predictable base-pairings, low-cost synthesis, biocompatibility, and specific interactions with wide range of biomolecules. The functional arrangement of unnatural peptides (Minchev and Sofroniev 1982; Mintchev et al. 1986) and synthetic oligonucleotides may be engineered as a platform for development of origami-based DNA assembles conjugated with aptamer or antibody ligands, i.e. programmed theranostics nanomachines, which are able to recognize and bind to the overexpressed membrane proteins on the target cells (Endo and Sugiyama 2018). In general, the most reported DNA-based nanomachines are nanoscale mechanical devices with high programmability due to their specific sequencing of nucleotide bases in the chain and the molecular recognition between complementary strands (Bath and Turberfield 2007). A variety of nanomachines have been utilized with potential application in biotechnology and nanomedicine research as nanorobots, nanotweezers, nanotransporters, nanowalkers, nanogears, etc. (Xing et al. 2017). Some of them are able to respond to specific molecular cues, to give a fluorescent signal through performing of a directional motion or release a cargo. For example, DNA aptamer-target interactions fabricate a series of "DNA nano-claws" (You et al. 2014) for autonomous and simultaneous analysis of multiple surface markers on cancer cell or reporting of mutated RNA inside cells. Despite their impressive diversities the most DNA-based nanomachines are usually triggered by a quite restricted environmental stimuli like temperature, light, or pH-change (Ranallo et al. 2017). In the case of cancer theranostics those nanomachines may be used as multi-input probes for both precise cancer cells recognition and stimulus-responsive carriers for controllable drug delivery (Qiao et al. 2018). The demonstration of their programmable performance at a molecular level as presented below is amazing and compelling.

Oligonucleotide aptamers for functionalization of nanomachines

The aptamers are single-stranded RNA or DNA oligonucleotides which have binding and selection affinity to distinguish cancer cells comparable to that of antibodies. They can covalently conjugate to small-molecule drugs (such as doxorubicin), unnatural peptides (Sofroniev and Mintchev et al. 1986; Minchev 1988), biotoxin or radioactive element tags and successfully target the cancer cells with simultaneously improved delivery efficiency and reduced side effects. The therapeutic aptamers may inhibit the activity of cellular receptors and dozens of them are already approved in clinical trials for synergistic treatment of tumors with conventional chemotherapy, thrombosis prevention or immune modulation (Zho and Rossi 2017). The multivalent aptamer complexes demonstrated significantly improved tumor retention pharmacokinetic abilities. Their other unique merits as excellent stability with low immunogenicity in the body, tissue penetration as well as the low cost of production make the oligonucleotide aptamers ideal molecular ligands for functionalization of theranostics nanomachines. As example, a programmed DNA origami structure of nanorobot has been reported with open/close switch. It was designed to deliver effective drugs to tumor and to respond to specific biomarkers (Li et al. 2018). The nanorobot is functionalized with DNA aptamers with aim to target and bind the expressed membrane proteins on the tumor-associated endothelial cells (Fig. 1). In the closed state the tubular-shaped nanorobot carry inside the antibody thrombin that is responsible for blood coagulation. When the aptamer reacts with the tumor associated target protein the tubular origami structure opens mechanically as a respond. Then the delivered inside thrombin is exposed to the blood in tumor-associated vessel and induce its massive coagulation at that site. The occurred necrosis inhibits the future tumor growth in the body. The "key" of the mechanism to lock or open the programmed nanorobot is the presence of functional DNA aptamers and their responding to external stimuli as specific biomarker. Such design can be used as a platform for the engineering of other nanomechanical devices (nubots) that respond to specific molecular cue.

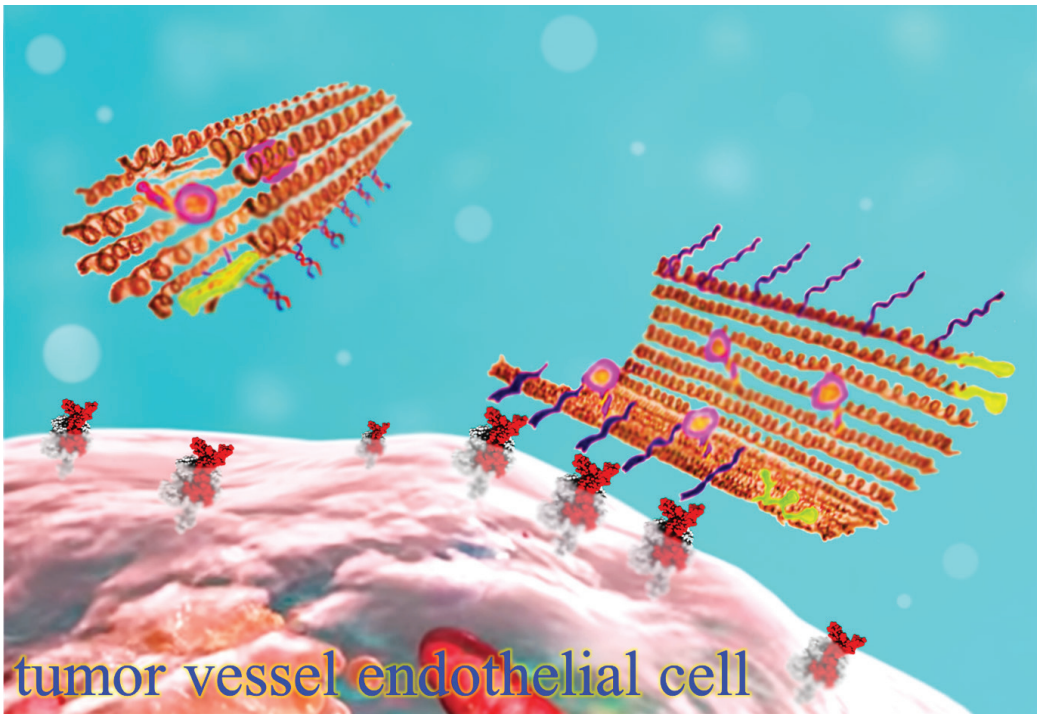

Figure 1. DNA programmed nanorobot functionalized with aptamers for targeting and binding to protein biomarkers expressed on the tumor-associated endothelial cells. The attached antibodies are the key for the mechanism to open the tubular-origami shape and performing of massive blood coagulation at the tumor site.

Programmable and multiparameter theranostics DNA-based nanomachine

A multi-parameter DNA-based logic platform have been designed as a base to develop programmable nanomachines for precise targeting and recognition of cancer cells through identification of high-order multiple markers (You et al. 2015). Their performance has been demonstrated through autonomous logic analysis of the abnormally expressed two or three biomarkers on the cancer cell surface owing to the three aptamers (sgc8, sgc4f and TC01), which are conjugated to the DNA platform (as shown on Fig. 2). The aptamers can be selected by the process known as cell-SELEX (Shangguan et al. 2006). Their function is to screen the presence of target biological markers as a different expression pattern of the membrane receptors on the cancer cells membrane. Thus, the designed nanomachine is able to distinguish various abnormal states of the tumor growth or even the population of same cells but at different stages of their physiological cycle. Such screening approach of the high or low expression levels of multiple membrane receptors enable the performance of intelligent tasks with more accuracy in respect to the screening of abnormal conditions on the cell surface. Once the cancer cell is discriminated the nanomachine can take its action. The binding between aptamers and target biomarkers is accompanied by the release of pre-hybridized cDNA, which further results in emission of a fluorescence signal. If the fluorescent dye is replaced by therapeutic reagent, this nanomachine could perform function of controlled drug release. It may also deftly survey the biological tapestry of cell surface, pinpoint its offending type, and use the cancer feedback mechanism to deliver the appropriate therapeutic drugs. The predictability of presented integrated multiligand profiling approach may prevent the extraneous target effects on normal cells and can be employed to construct of smarter nanodevices with biomedical application for diagnosis and therapy of cancer diseases.

Antibody-powered DNA nanomachine

A new class antibody-powered DNA-based nanomachine was demonstrated for controlled drug-release, point-of-care diagnostics, and *in vivo* imaging application (Ranallo et al. 2017). Its design has been inspired from the function of the transport proteins that can load and release molecular cargo through regulated conformational change of a triplet complex. By mimicking this mechanism, the developed DNA-nanomachine is programmed to reversibly load and release a molecular cargo (e.g., an oligonucleotide strand) in a highly stable fashion through specific binding with antibody. As shown on Fig. 3 the design strategy of this nanomachine takes the advantage of DNA sequences to form a triplex with another shorter oligonucleotide strand by involving Watson-Crick (–) and Hoogsten (●) interactions. Thereby the formation of clamp-like structure has been achieved and the longer fiber chain is conjugated with a pair of antigens at 5’ and 3’-ends, respectively. When the

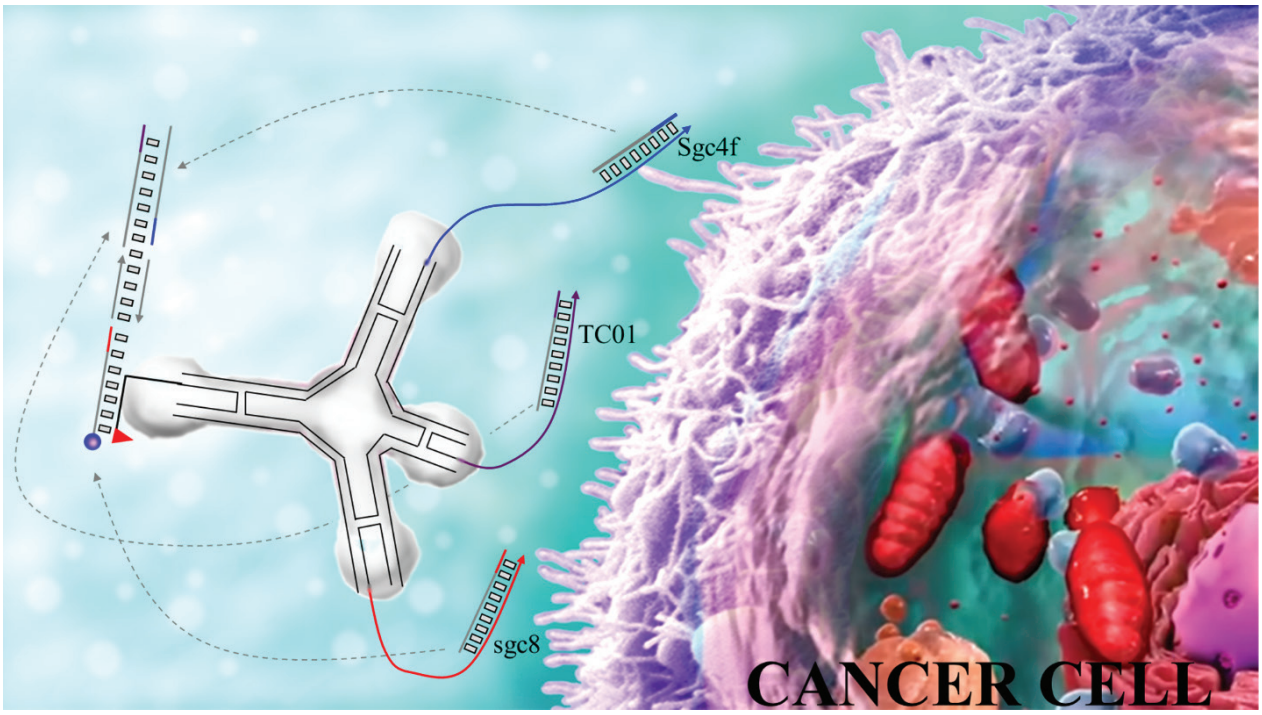

Figure 2. Schematic illustration of the programmed operation of multiparameter theranostics DNA-based nanomachine for logic-based autonomous targeting and therapy of cancer cells.

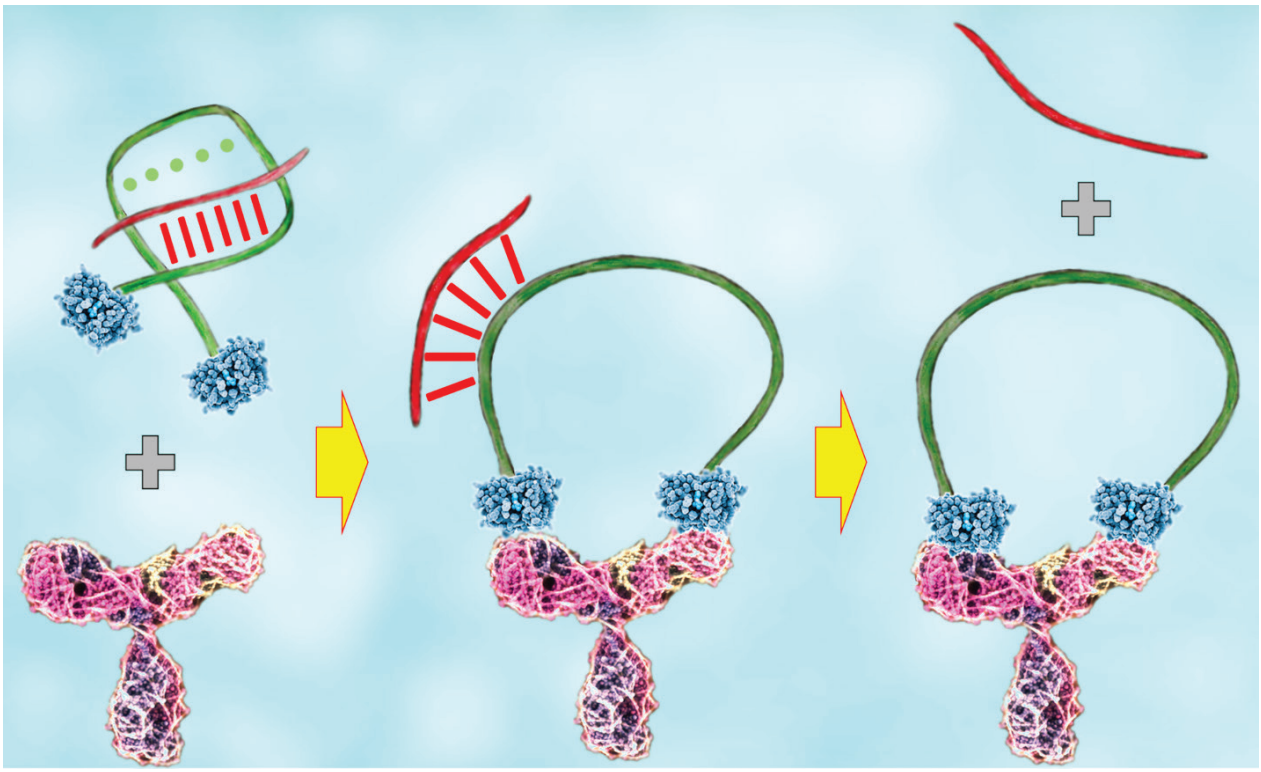

Figure 3. Principle of conformational change and induced triplex opening of the antibody-powered DNA nanomachine.

nanomachine's antigens are bound to the target antibody as bidentate immobilization, the triplex construction is opening, and the contained cargo (the red oligonucleotide fragment) is released away from the formed complex. The reason for cargo release is because the initially formed Hoogsteen interactions within the triplex-complex in the engineered design are energetically disrupted and easy to destabilize as in the case of bidentate binding with the antibody. The induced conformational change is in similar analogy as the antibody triggered stem-loop opening (Ranallo et al. 2015). Moreover, obtained data revealed that the amount of oligonucleotide cargo and its rate of releasing are proportional to the concentration of the applied antibody in the experimental setup. This suggest

that the binding process must be the rate-limiting step of the proposed cargo-release mechanism in the antibody-powered nanomachine. The principle of presented design is highly generalizable. So, it can be easily adapted to any other system which employed antibody or unnatural peptides (Sofroniev et al. 1981; Mintchev et al. 1985) for specific interaction with antigen-terminated DNA that initially are prepared in triplex fiber structure. The use of antibodies triggering inputs to release a specific molecular cargo may open new opportunities for application of these nanomachines as clinical and diagnostic markers.

Nanozyme-based nanomachines

By the end of 2020 about one thousand nanomaterials have been reported to mimics the mechanisms and biofunctions of natural enzymes. They are called nanozymes. The nanozymes possess unique properties, which provide ideas and platform for creative designs and fabrication of novel theranostic nanomachines. For example, the carbon nanodots-based nanozymes may successfully simulated the structure and function of natural enzymes such as oxidase (Loukanov et al. 2022), catalase and superoxide dismutase (Zhao et al. 2020). The carbon nanodots are produced from various organic molecules as sugars, proteinogenic amino acids (e.g., histidine), or unnatural amino acids (Mintchev et al. 1990; Sofroniev 2007a, b, c, 2008a, b, 2009a, b, 2010a, b, 2011a, b, 2012), which enable the nanozyme to target and treat tumor cells or detect biomolecules *in vivo* or *in vitro*. For example, if the particles are decorated with tosyl groups they are able to capture cancer cells and deliver DOX. If decorated with targeting ligands, nanozymes might be applied in motion control of the nanomachine and utilized in its „ON" and „OFF" logic control system. The exome-like nanozyme vesicles are used for clinical cancer diagnosis (Ding et al. 2018). The graphene quantum dot nanozyme form vesicles in the natural erythocyte together with 3-ethylbenzothiazoline-6-sulfonic acid. If the surface of this nanomachine is modified with folate acid, it might target nasopharyngeal carcinoma cells thanks to the reaction of recognition with their overexpressed folate receptors. The nanomachine based on quantum dot nanozyme acts as theranostic agent because it can be applied in the photoacoustic imaging and simultaneously destroy the cancer tissue by ROS (reactive oxygen species) under near-infrared photodynamic therapy. Based on the ROS damage it was designed another multi-nanozyme nanoflowers-machine, which was able to kill the tumor cells too (Wang et al. 2018). The nanoflowers combined two types' nanozymes – PtCo with intrinsic oxidase-like activity, and MnO_2 with catalase mimicking activity. The purpose of MnO_2 is to convert the high level of H_2O_2 in tumor cells to O_2, which is involved in cascades of oxidation reactions catalyzed by the PtCo nanozyme. As result, apoptosis and necrosis is induced in the cancer cells. Moreover, the nanoflowers-machine does not need external supply of oxygen, and it showed little cytotoxicity towards the healthy tissues. Thus, it can be applied in the practical chemotherapy.

Other multishape nanozymes have also been designed for therapeutic applications, such as core-shell, Janus, yolk-shell, bowl-like or alloy structures. Janus nanomotors are defined as asymmetrical particles with various physical or chemical properties on different hemispheres. Multifunctional Janus nanomachines provide synergistic effects by concentrating multiple properties on a single carrier (Loukanov 2021). They have become a research hotspot, especially in the field of biomedicine and theranostic research, owing to their diversity in composition and asymmetry in structure, surface selective modification, as well as host drugs with different solubility in their distinct domains.

Nanomachine for recognition and cleavage of specific RNA

A theranostic DNA nanomachine has been designed, which can simultaneously target, report, and cleave specific RNA at conditions near to the physiological one (Spelkov et al. 2020). The presented strategy is developed on a single double-stranded theranostic DNA platform, in which oligonucleotides as four arms have been attached in order to accomplish simultaneously therapeutic and diagnostic functions in respect to the target RNA (Fig. 4). The arms 1 and 2 operate as deoxyribosome Dz1, which bind the specific site of RNA through base pairing (i) and catalyze a reaction 1 of cleavage and deactivation. Thus, the therapeutic effect of nanomachine is achieved (ii). The arms 3 and 4 act as binary deoxyribozyme Dz2 and cooperatively bind both the target RNA and another molecular reporter (or reaction 2) known as fluorophore (F) and quencher (Q) labeled fiber oligonucleotide (iii). The opening of fiber structure results in fluorescence signal increasing (reaction 3), which is indication for the programmed diagnostic function (vi). The nanomachine' arms have appropriate length, which enable the complementary binding with the nucleotide bases of RNA chain. The short RNA-binding arms enable enhanced affinity of the nanomachine to recognize the specific RNA. Thus, high selectivity may be achieved (Smith and Kolpashchikov 2017). Their programmed cooperative functioning allows efficiently unwind of the secondary structure of RNA, as well as its further cleaving. Such DNA-based nanomachine may find great application in the treatment of genetic disorder and cancer diseases or diagnostics and therapy of viral infections, since the intracellular recognition and destruction of mutated RNA is of high importance. The possibilities to destroy specific RNA could create also new algorithm approaches in the personalized nanomedicine.

Gold nanomachine for photoacoustic imaging and photothermal therapy

The assembly of gold nanoparticles (Au NPs) through dynamic DNA-fueled molecular machines can be powerful platform for developing of theranostics medication. In such nanomachine, the aggregation of DNA-functionalized Au NPs may be regulated by a

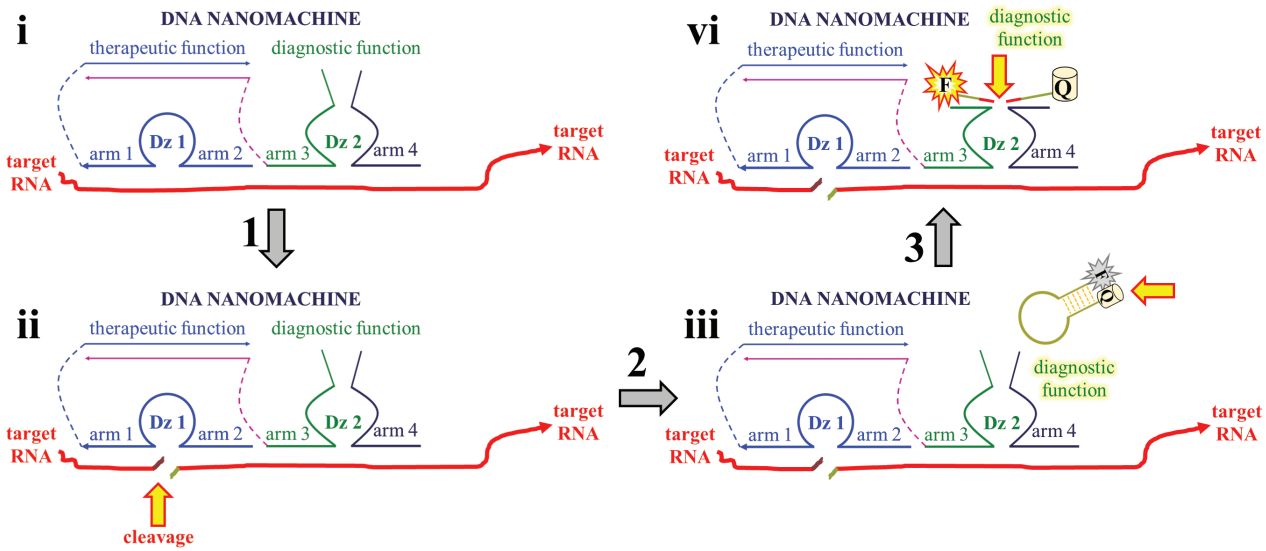

Figure 4. Design of the theranostic nanomachine for targeting and cleavage of RNA. *Reaction 1*: the nanomachine strand with Dz1 catalytic core (deoxyribozyme) bind to a specific site of the target RNA by Watson-Crick base pairing and cleave the chain. *Reaction 2*: a binary sensor hybridizes in complementary approach to form Dz2 core, which is able to react with a fluorescent reporter. *Reaction 3*: Dz2 core cleaves the fluorescent reporter and induces a fluorescent signal with increased intensity.

series of strand displacement reactions. The DNA-linkers provide connection between the individual Au NPs and thus numerous nanodevices with unique properties and functions might be fabricated. Such innovative design of a theranostic nanomachine made from gold nanoparticles conjugated to single-stranded DNA (ss DNA) oligonucleotides have been reported (Yu et al. 2017). Its anticancer performance as well as excellent therapeutic effects with low systemic toxicity has been demonstrated. For that purpose, the equally sized gold nanoparticles (Au NPs) were coated with pyridine-2-imine-terminated single-strand DNAs through gold-thiol bonds. However, at that step the equally-sized Au NPs were preliminary split into two groups and each of them was coated with complementary to each other ss DNA strands. Thus, the separated nanoparticles exist well dispersed in both samples, but when mixed their complementary DNA strands hybridized and formed large-sized Au NPs aggregates (as shown on Fig. 5A). To prevent the hybridization process of the mixed Au NPs the pyridine-2-imine on their DNA ends were capped with alpha-cyclodextrin (α-CD) moieties via noncovalent electrostatic interactions (as shown on Fig. 5B). This process is reversible, and it is pH-dependent. Therefore, the obtained two types (α-CD)-based gold/DNA nanomachines (abbreviated as Au-DNA- αCD) have interesting physicochemical properties and their release is triggered by stimuli as the alteration of pH in the tumor microenvironment (as shown on Fig. 5C). When injected into the blood vessels of experimental mousses, the Au-DNA- αCD possessed high nuclease resistance and avoided the clearance during blood circulation. Their high stability under physiological condition is a critical factor to accumulate in the tumor with maximum extent. Once the nanomachine reach the tumor region via enhanced penetration and retention it is subject of altered pH (from physiological pH 7.4 to 6.5), which activates its programmed functions. At this slightly acidic environment the pyridine-2-imine are going to protonated form and the non-covalent electrostatic forces are reduced. As a consequence, α-CD moiety is immediately separated from the oligonucleotide end and the hybridization between complementary strains occurred. Subsequently, Au NPs formed aggregates, which remain in the tumor tissue against the high interstitial fluid pressure (same as Fig. 5A), thereby realizing the mission of tumor-specific targeting. The *in vivo* results indicate the Au NPs aggregates have high near-infrared adsorption capacity and serve as efficient theranostic agents for simultaneously photothermal therapy and photoacoustic imaging.

In the clinical application it must be taken under consideration the stability of the oligonucleotide chain against the presence of deoxyribonucleases enzymes (DNases). DNases might degrade the "intruder" DNA and thus abolish the functionality of the nanomachine (Toshev et al. 2020). Therefore, the protection of the oligonucleotide linkers by chemical modification, packaging or using DNase inhibitors could be a necessary step in order to allow the nanomachine to realize its designed operation.

Conclusion

The promising development in nanotechnology-based theranostics can completely change our targeted drug delivery approach. Over the past decade, significant research efforts have been focused on the design of nanomaterials whose properties and, therefore, behavior is regulated in a programmable fashion. Nowadays, the highly programmed nanomachines are able to identify the challenging cellular targets and to release drugs in

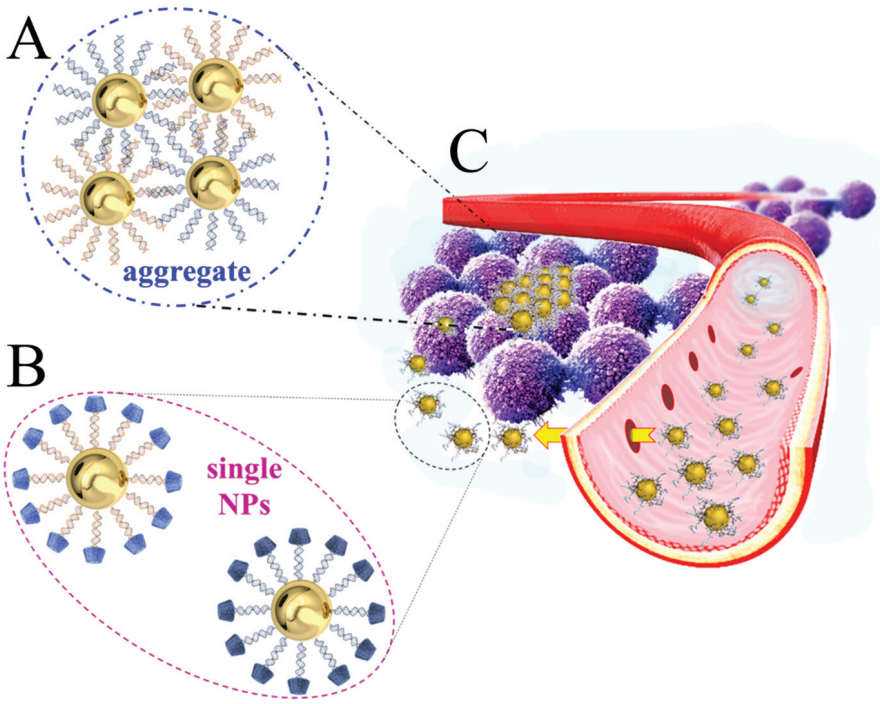

Figure 5. Schematic illustration of: (**A**) aggregate formation of gold nanoparticles coated with complementary ss DNA strands; (**B**) single dispersed gold nanoparticles coated with ss DNA strands and protected with alpha-cyclodextrin to avoid aggregation; and (**C**) tumor-specific photoacoustic imaging and photothermal therapy.

a precisely regulated fashion, as well as to broadcast the information about the local microenvironment. In this respect, there is a great interest and significant development progress of those task-specific nanodevices into an all-in-one theranostic platform to afford powerful nanomachines that surpass all state-of-the-art drugs.

Acknowledgements

The authors are thankful to MEXT/JSPS KAKENHI Grant Number 20K05260 and Jikoshunyu Kyoin-haibun-keihi № T5452 projects for the funding of our research in the field of nanomachines and nanorobots.

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