JOURNAL OF PHARMA INSIGHTS AND RESEARCH

REVIEW ARTICLE

DNA Nanorobots In Cancer Therapy- A Review

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Publication history: Received on 21st October; Revised on 18th November; Accepted on 22nd November

Article DOI: 10.5281/zenodo.10232012

Abstract: DNA nanotechnology facilitates the utilization of programmable nanorobots in-field applications of biomedicine. Designer nanorobots constructed from DNA offer a wide range of functionality in both biosensing and the delivery of pharmaceutical agents. The utilization of DNA nanobots has been identified as an increasingly safe and precise approach for the treatment of cancer. These nanobots are specifically engineered to locate and eradicate malignant cells. DNA nanotechnology serves as a tool for the creation of functional nanostructures. Nanostructures constructed from DNA possess a multitude of uses in the realm of synthetic biology. DNA nanobots represent a potential technique for the treatment of cancer. These nanobots possess the ability to selectively target and eliminate cancerous cells. DNA nanodevices have produced remarkable advancements in the realm of cell biology. DNA can be employed in the construction of nanomechanical devices. Numerous DNA machines have been designed and subjected to experimental testing. Nanobots are currently under investigation as potential systems for the delivery of pharmaceutical agents. These nanobots exhibit great promise in the identification and management of various diseases. Developments in the are of nanorobotics have expanded the realm of medical robotics. Biomedical applications of nanorobots span across various domains such as diagnosis, sensing, microsurgery, the delivery of pharmaceutical agents and cells, and wound healing. This review will explore the construction and utilization of DNA nanodevices in cancer treatment.

Keywords: DNA nanorobot; Nanotechnology; DNA origami; Cancer therapy; Targeted drug delivery

1. Introduction

Cancer is a prevalent global public health concern and the most prominent cause of mortality subsequent to cardiac disease. Cancer, an affliction universally indiscriminate, manifests its pernicious influence upon individuals from all walks of life, irrespective of wealth or age. It is believed that the appellation "cancer" was bestowed upon this malady by the venerable Hippocrates. Intriguingly, the ancient physician postulated the existence of four bodily humours, namely blood, phlegm, yellow bile, and black bile, with an excess of the latter being implicated in the development of cancer [1].

There exist over 200 cancer types, which researchers categorize based on their origin location. The four primary cancer types are carcinomas, sarcomas, leukaemia, and lymphomas. Genetic mutations can be classified into two fundamental categories: acquired mutations and germline mutations. In approximately 5-10% of cancer cases, gene mutations inherited from previous generations significantly predispose individuals to this ailment [2]. At present, a plethora of cancer treatments are available and their selection is contingent upon the type and stage of the cancer. While some patients may solely require one treatment modality, others may necessitate a combination of treatments such as surgery, chemotherapy, medication, or radiation therapy, which can be administered in various combinations and at different times.

Chemotherapy can elicit a spectrum of adverse effects, which can vary depending on the specific medications employed and the individual's response to treatment. Frequently encountered adverse effects of chemotherapy include nausea and vomiting, hair loss, fatigue, loss of appetite, alterations in taste and smell perception. Chemotherapy can also lead to decrease in red blood cells (anaemia), an increased susceptibility to infections due to decreased white blood cell counts, and an augmented risk of bleeding due to reduced platelet counts. Additional potential side effects encompass mouth ulcers, diarrhoea or constipation, peripheral nerve damage (peripheral neuropathy), fertility complications, fluctuations in mood or cognitive function. It is crucial to understand that not everyone will experience the same side effects, and the severity of these adverse events can differ [3].

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The past few years have witnessed the utilization of various materials, including self-assembled polymers and metal nanoparticles, for the evolution of nanostructures that aid in the delivery of anticancer drugs. This development represents a notable advancement in the realm of cancer therapy. Specifically, drug delivery systems based on nanoparticles have demonstrated the ability to enhance permeability and retention (EPR) effects, resulting in the accumulation of drugs in the tumour region through passive means. Despite significant efforts being devoted to the development of various materials for use as carriers at the nanoscale, the primary challenge in chemotherapy administration remains the construction of safe, biocompatible, and effective carriers for drug delivery within living organisms [4].

2. Building a DNA Nanorobot

2.1. Strategies

In biological systems, DNA is a hereditary material in humans and almost all other organisms. DNA nanotechnology takes this molecule from its biological setting and utilizes its understanding to build structural motifs and then connect them together [5].Approaches used to construct DNA nanostructures include tile based construction, origami based construction, nanoparticles templating, metal hybridization and many more. The exceptional characteristics of molecular recognition and the capacity to create complementary base pairs, combined with longevity, nanoscale repeating units, and the ease of use of customizing strand lengths to suit specific requirements, facilitate the construction of a wide assortments of nanostructures. Furthermore, the effective absorption by cells and the elevated effectiveness of drug encapsulation establish DNA origami is an optimal "intelligent" foundational element, possessing programmable potential in the generation and enhancement of versatile, secure drug delivery systems [6].

2.2. DNA Origami

The art of creating small, precisely defined shapes out of deoxyribonucleic acid (DNA) is known as DNA origami. [9]An important turning point in the development of DNA nanotechnology was reached with the creation of DNA origami. This innovative method has several clear benefits. To begin with, origami constructions have a distinct shape. Secondly, sequence optimization is usually not needed in the process of building a desired DNA origami structure [7]. In fact, once the folding process is discovered, the sequences of the staple strands are preset because M13mp18 genomic DNA is used to make most origami designs. Many modeling software packages are currently available to assist users in creating origami structures. Sequences for hundreds of staple strands can be automatically created and exported as a single file by describing the scaffold pathway and its starting point. This makes subsequent chemical synthesis simpler. For newbies and anyone interested in DNA nanotechnology in particular, the DNA origami technique makes design and assembly more convenient. Thirdly, raw, unpurified core strands are suitable to create DNA origami. Typically, the molar ratio between staple strands and the M13mp18 long strand is 10 to 1 or less. Moreover, the yield of the target shape is usually higher than 90%. All of these features combine to make DNA origami an effortless but effective design method that promotes and accelerates the advancement of DNA nanotechnology. It is important to remember that the core ideas and concepts of DNA origami—namely, the utilization of crossovers to connect or bundle DNA duplexes and create nanostructures—remain unchanged. The origami technique generates several crossovers at every place inside the area that the M13mp18 scaffold provides. The well-defined nature of origami structures may be due in part to the packing of many duplex domains and the high density of crossovers [1,8].

Some typical DNA nanostructures constructed from DNA origami include:
(i) the smiley face icon, 3D DNA boxes and Nano flasks
(ii) 3D DNA tetrahedron
(iii) DNA origami dolphin.

2.3. Aptamer integrated nanostructures

DNA aptamers are oligonucleotides with a single strand that are screened using an in vitro technique called as systematic development of ligands by exponential enrichment (SELEX), which was first developed by the Gold and Szostak Labs in 1990. These aptamers, often referred to as "chemical antibodies," have the power to specifically and strongly bind to many targets, including metal ions, small molecules, proteins, cells, viruses, and bacteria. In comparison to antibodies, aptamers offer several additional advantages, such as their relatively small size, greater level of design flexibility, ease of modification, and low potential for immune response. These distinctive characteristics make aptamers highly promising tools for recognition in biological and biomedical research [9]. Furthermore, when combined with DNA nanotechnology, aptamers can be utilized to create adaptable and tailored Nano systems that possess exceptional recognition capabilities and can respond intelligently to a growing array of bio targets. Hence, the integration of aptamers with DNA nanotechnology holds great potential in the realms of biology and biomedicine.

2.4. Synthesis of Aptamers

Aptamers can be screened from a library composed of many oligonucleotides by a process known as systematic evolution of ligands by exponential enrichment. (SELEX)

Gold28 and Szostak29 separately created the SELEX technique in 1990. In conclusion, the general SELEX procedure includes incubating the target of interest with the oligonucleotide library pool (DNA). After washing, the bound sequences are eluted, and if negative selection is required, they are incubated with a control target to remove sequences that also show recognition to the control. Following that, PCR is used to generate the remaining sequences. Until the pool's specific binding intensity to the target reaches an appropriate level, this process is repeated. To acquire individual sequences, the enriched pool is cloned and sequenced. Samples of these sequences are then chemically produced, sensors are added, and the samples are assessed against the target to ascertain potential candidates [10].

Aptamers were produced originally in the SELEX development process to target simple targets like tiny compounds and pure proteins. Afterwards, it was shown that aptamer selection could be used to complicated targets, especially live cells (cell-SELEX). Cell-SELEX has an important benefit over protein-based SELEX in that it can isolate aptamers against cancer cells without knowing the quantity or kind of proteins on the cell surface beforehand. This makes it possible to obtain aptamers that only attach to a certain kind of cancer cell and do not attach to healthy cells. To further facilitate the production of many aptamer sequences in a single selection, selection is carried out against entire cells that express various protein receptors on their membrane. Therefore, cell-based SELEX offers a useful technique for locating putative disease indicators for the detection and management of cancer. The targeted delivery of therapies to cancer cells and the early identification of cancer cells or cancer-related indicators can both be aided by these aptamers [11].

2.5. Construction of Aptamer integrated nanostructures

Aptamers can be integrated into DNA nanostructures either through base pair hybridization between aptamers and DNA nanostructures or as a component in the assembly procedure. In general, there are two main approaches for the preparation of aptamer integrated DNA nanostructures namely, nucleic acid hybridization-dependent assembly and non-hybridization-dependent assembly [12].

The essential characteristics of aptamers that necessitate optimization for the purpose of drug development encompass substantial affinity and specificity, as well as an extended half-life within the pertinent biological compartment. [7] Aptamers have already demonstrated success in accurately and selectively identifying specific types of tumour cells or tissues as effective targeting agents. The integration of nanomaterials with aptamers in bio conjugates will expedite the advancement of efficient cancer treatment methods. The use of aptamers in conjunction with organic and inorganic nanomaterials offers significant advantages in drug delivery due to their resistance to degradation in biological systems. The integration of aptamers with Nano medicine has established promising objectives for the control and suppression of cancer. Several aptamers based on SELEX methodology exhibit clinical potential as standalone therapies or in combination with conventional chemotherapeutics for treating various forms of cancer. Additionally, the incorporation of aptamers in new drug delivery techniques has enhanced their target-specific therapeutic capabilities. Consequently, the combination of aptamers, with their distinct structural characteristics, may facilitate the development of novel cancer treatment approaches. Despite considerable progress in tumor application research, there is still a need for improvements in the amount of medicine loaded, targeting precision, circulation duration, and affinity of aptamers, among other aspects. It is important to acquire a deeper knowledge of the interactions between aptamers and medications, as well as the impact and modification of nanocarrier properties by aptamers in order to address the limitations associated with aptamers, their conjugation with nanoparticles, loading capacity and circulation duration of the nanocarrier. Thus, the combination of nanoparticles and aptamers as individual components results in a synergistic effect and mutually beneficial functionality. Furthermore, aptamers have been successfully conjugated with various types of nanoparticles, including both organic and inorganic nanoparticles. We anticipate that aptamers will assume a more significant role in cancer therapeutic applications in the future, as SELEX technology and various synthesis techniques continue to advance and gain widespread implementation [13].

3. Clinical Evaluation

In recent years, a number of Nanodrug delivery systems modified with aptamers have been developed to enhance therapeutic effectiveness and minimize undesirable side effects. For instance, Lin and his colleagues engineered a nanomedicine by incorporating DNA aptamers (AS1411) and an antimetabolite drug (5-fluorouracil [5-FU]) into DNA tetrahedra. Through investigating the differences in cellular uptake between breast cancer cells and normal breast cells, the researchers discovered that this cancer-targeting nanomedicine (AS1411-T-5-FU) exhibited superior therapeutic efficacy on the target cells compared to free 5-FU [14].

Doxorubicin is extensively and commercially utilized in the realm of cancer therapy, particularly for solid tumors. Its characteristic as a well-established DNA intercalator further adds to its prominence. Hence, in principle, it possesses the potential to be among

the most auspicious contenders for DNA Nanotechnology based delivery. This is due to its capability to be incorporated into tailored nanostructures that could potentially offer a multitude of additional functionalities. [10] In addition to their targeted drug delivery capabilities, nanobots can also combat tumors by impeding the flow of blood to the tumor cells. As tumor cells are reliant on a steady supply of blood to survive, scientists have focused their efforts on exploiting this vulnerability. The nanobot designed for this purpose is made of a flat DNA sheet to which an enzyme called thrombin, responsible for blood clotting, is attached. This sheet is then rolled into a tube, with thrombin encapsulated within it. DNA aptamers are affixed to the surface of the tube, which are capable of specifically recognizing and binding to a protein called nucleoin, found exclusively on the surface of cancer cells and not healthy cells. The DNA aptamers are then able to locate and attach themselves to the surface of the cancer cells. Subsequently, the nanobots penetrate the blood vessels that supply the tumor and, upon unrolling, release the thrombin molecules. This initiates the clotting process, leading to a reduction in blood flow to the tumor and ultimately starving the tumor of vital nutrients [15].

The successful diagnosis and treatment of cancer can be achieved through the utilization of nanobots. In comparison to traditional drugs, nanobots possess a remarkable level of specificity, as they are programmed to exclusively identify and interact with diseased cells, while leaving healthy cells untouched. Consequently, the occurrence of adverse effects is minimized. By incorporating biosensors into nanobots, the early stages of tumor cell development within a patient's body can be detected [16].

4. Bacteriobots Vs DNA Nanobots

Scientists have taken the approach of genetically modifying salmonella bacteria to transport miniature robots, known as bacteriobots, which are drawn to tumors due to the chemicals released by cancerous cells. These bacteriobots effectively deliver drugs directly to the tumor, while sparing the healthy cells, thereby shielding the patient from the side effects commonly associated with chemotherapy. However, it is vital to note that these bacteriobots are only capable of detecting breast cancer and colorectal cancers. On the contrary, nanobots are capable of detecting and treating various other types of cancer. Moreover, nanobots can be engineered to target specific cell surface receptors, and the therapeutics they release upon activation can be tailored to meet the specific requirements of each case. The establishment of nanobots involves the usage of engineered DNA strands that have been meticulously designed to fold into a desired tertiary structure. Once bound to the desired target, the conformation of the DNA nanobot undergoes a transformation from a closed state to a tertiary state, facilitating the release of the stored therapy [16].

5. Advantages of DNA nanorobots

Following are the advantages [5,13] of DNA Nanorobots:

- The primary advantages of the bots are their speed and durability. Benefits of DNA nanobots in delivering drugs exceed those provided by present approaches.
- Additionally, there is regulated drug release, which is very particular and accurate, and has less negative effects. It reduces surgical errors. Its superiority is due to computer-controlled distribution and the quicker treatment effect.
- By performing superior biomedical therapies using minimally invasive procedures, nanorobots improve treatment efficiency.
- Nanorobots could assist with early tumor diagnosis and drug administration via intelligent chemotherapy.
- Nanorobots as drug carriers for chronic delivery regimens may allow chemical substances to stay in circulation for a longer duration of time, depending on the situation.

6. Challenges of DNA nanorobot based targeted drug delivery

The challenges[10,12] of DNA nanorobot based targeted drug delivery are:

- The possible immunogenicity of these nanorobots is a key obstacle to any serious execution of this technology in humans.
- Another issue to be concerned about is the stability as well as structural integrity of DNA nanostructures inside the bloodstream, the presence of nucleases and a low salt concentration have previously been shown to have a major effect on the stability and circulation time of nanostructures.
- However, methods for coating and ionic stabilization could help remedy this problem.
- The current nanobots primarily serve as drug carriers, and their movements are dependent on blood flow. Once the medicine within is delivered at the target, the nanobots are eliminated by the body's immune system. To broaden the uses, nanobots must be reused, and communication and coordination among nanobots within the body is required.
- Nanostructure assembly and off-target issues are significant challenges in usage of DNA nanobots in cancer therapy.
- According to research, the blood-tumor barrier varies considerably from the healthy blood-brain barrier. Tight junction opening and increased malignancy are the most significant problems. As consequence, gliomas (brain cancer) lack the

expression of a non-functional version of occludin. In this case, BBB causes tumor accumulation of various nanocarriers, which has a substantial influence on the outcome of nanocarriers aimed at treating brain cancer.

7. Conclusion

DNA nanobots have got incredible demand in research to be utilized in the targeted drug delivery especially in cancer treatment. Various theories have been set up and different techniques for the synthesis of DNA nanobots are under study. All the concerning challenges are being reviewed and necessary modifications are being made by the scientists. The structural aspects of DNA make it most useful to be used as a drug carrier. In the future, medical treatment based on nanotechnology will expand enormously, especially in the management of various cancers.

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