USING NANOPARTICLE-APTAMER BIOCONJUGATES FOR IMAGING AND TREATING PROSTATE CANCER

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ABSTRACT
Prostate cancer diagnoses increase each year, and current treatment strategies cause disturbing levels of serious side effects. This has necessitated a search for new strategies to employ more targeted treatments for malignant tissues. One promising alternative therapy is the use of a chemotherapeutic-nanoparticle-aptamer bioconjugate. This method employs aptamers which target over-expressed proteins on cancerous cell surfaces and bind to individual prostate tumour cells with incredible affinity. Once bound, the bioconjugate is taken into the cell where it delivers a toxic payload of chemotherapeutics and destroys the cell by cytotoxic means. The bioconjugate therapy method is specific for cancerous cells which limits side-effects to non-target tissues. Fluorescent properties of some chemotherapeutic components and quantum dot nanoparticles can also provide imaging of these cancerous masses with extreme precision. Successful trials employing aptamers for targeted therapy demonstrate the promise of this technology for future chemotherapeutic applications. Additionally, aptamer conjugates are safer, less expensive, and potentially more effective substitutes to antibody-based targeting methods which are currently being explored as a competing option for this type of treatment.

INTRODUCTION
Of the approximately 100,000 cases of male cancer diagnoses in Canada each year, 23.9% of these diagnoses will be for prostate cancer, which makes it the most prevalent cancer among Canadian men[1]. Although methods have been developed to successfully detect and treat prostate cancer initiation and progression, there are several detrimental side-effects that occur at high rates when using these strategies. Some of these effects include impotence, which occurs in approximately 43% of patients, urinary retention in 24% and radiation-induced bowel injury in 1%. Another commonly employed treatment strategy is the full removal of the prostate gland which results in even higher rates of impotence as well as urinary incontinence[2]. It is therefore highly desirable to find an alternative which can effectively detect and treat prostate cancer while limiting the side effects associated with current strategies.

One promising, less harmful alternative to the traditional therapeutic strategies, is the use of aptamer based therapeutic conjugates. Aptamers, developed in 1990 and named from the Greek aptus meaning ‘to fit’, are small pieces of DNA or RNA that can fold into different 3D conformations...
and bind to a desired target with extreme specificity. Aptamers have demonstrated the ability to bind with virtually any target and to trigger responses upon the occurrence of correct aptamer-target interactions\[3\]. The intramolecular forces of an aptamer are unique to each and depend on the sequence of bases and chain length. These forces permit the aptamer to fold and twist into shapes that can bind to a variety molecular targets, including large proteins, cells, metal ions, and even viruses or parasites\[4\]. Due to these applications, aptamers draw many comparisons with antibodies. However, aptamers can bind with much higher specificity, are more cost effective, and can be constructed in vitro, negating animal use.

Aptamers are created using the systematic evolution of ligands by exponential enrichment (SELEX) process, which works by exposing a library of randomly generated oligonucleotide sequences, typically 10^{14} to 10^{18} sequences, to a target and isolating the ones that interact with some degree of affinity. The sequences which do not bind are washed away and discarded. The aptamers that bind are repeatedly subjected to the target under different conditions to increase stringency in the selection process. Alternating elution solvents, changing temperature, or decreasing target concentrations are all employed until a few aptamer sequences are isolated that bind to the target with incredibly high affinity\[5\].

Aptamer technology showing the greatest promise as a prostate cancer therapeutic involves a collaborative strategy in association with a chemotherapeutic drug, such as doxorubicin or docetaxel and a nanoparticle like a quantum dot (QD). The end product is called a bioconjugate, which is simply a compound made up of different molecules covalently bound together. Each element of the bioconjugate plays a significant and independent role. For example, an aptamer can act as a drug carrying vehicle and target individual cancerous cells by binding to the prostate specific membrane antigen (PSMA) protein. PSMA is a membrane protein which is over-expressed on the surface epithelial cells of prostate tumours\[6\]. Once the bioconjugate is bound, uptake into the cell is triggered via endocytosis. Depending on other components of the bioconjugate, it can serve several purposes once inside the cell including signalling, imaging and cytotoxicity. Moreover, the therapeutic destroys the cell and the nanoparticle provides fluorescent light emission and allows the cytotoxic cargo to be transferred safely by encapsulating it, thus protecting it from nuclease degradation\[9\]. The bioconjugate therefore not only delivers a targeted dose of chemotherapy but can also be used to image the tumour mass by utilizing its inherent fluorescent properties\[7\]. Aptamer targeting mechanisms and the wide variety of multipurpose conjugates that can be constructed using aptamers is showing significant promise to be one of the safest and most effective future developments in medicine.

**SYNTHESIS**

The first step in producing a therapeutic aptamer for a bioconjugate involves the isolation of a desired target. For the example presented in this paper, the desired target was PSMA, as it is found in abundance almost exclusively on prostate tumour cells. Lupold et al. (2002) isolated PSMA by identifying and culturing the cancerous cell lines from prostate tumour (LNCaP cells), isolating PSMA DNA found within the cells and preparing recombinant xPSM-expressing Baculoviruses. The isolated target was subsequently used for in vitro selection of aptamers that would bind via the SELEX process for nine rounds. By the sixth round of selection, 95% of the aptamers were sequences xPSM-A9 and xPSM-A10. The xPSM-A9 aptamer bound non-competitively to PSMA, and altered the active site, while the xPSM-A10 aptamer bound competitively, directly to the PSMA active site, which made it a more useful option for bioconjugate delivery. Afterwards the aptamer was selected and further modified with the addition of 2'-fluoro-pyrimidines to increase its stability in biological fluids, through the prevention of nuclease degradation\[6\].

Because aptamers are made of naturally occurring molecules, they a have a limited half-life in vivo, due to nuclease degradation and natural excretion. Modifications such as oligonucleotide terminal caps, cholesterol, and non-deoxyribose sugars can be added in order to protect the aptamer and increase half-life exponentially within the blood stream\[9\].

The second major part of the bioconjugate provided as an example is the quantum dot nanoparticle. QDs are semiconductor nano crystals that are often used in biological imaging, as they are fluorescent...
in nature and immune to chemical degradation. The QD and xPSM-A10 were then amalgamated creating a QD-aptamer conjugate with the aptamer acting as an escort for this system. The chemotherapeutic chemical, doxorubicin, which is also a fluorescent molecule, was then intercalated between the single 5’-CG-3’ regions of the 57 base pair xPSM-A10 aptamer sequence. When the doxorubicin loaded aptamer interacts with the quantum dot, it becomes a chemical beacon to signify the delivery of the chemotherapeutic payload. The florescence of the QD is “turned off” from a quenching interaction between the gold of the QD and the doxorubicin molecule. After the doxorubicin is released, the quenching interaction no longer exists and the QD florescence “turns on”[2,5].

APPLICATION

Once administered into the body, the bioconjugate interacts with PSMA proteins on the cell membrane of cancerous cells and binds with affinity and specificity. Upon binding, the entire bioconjugate is absorbed into the cell via endocytosis. Once inside the cell, doxorubicin is released from the aptamer through physical dissociation from the conjugates or from biodegradation of the aptamer by the cell’s lysosomal enzymes[7]. After the doxorubicin is released from the intercalated position in the aptamer, it is now free to kill the cell by cytotoxic mechanisms. In addition, the QD’s florescence is turned “on” as the fluorescence of the bioconjugate is no longer quenched by the doxorubicin. The light emitted from this nanoparticle can be captured by biological imaging devices to enable observation of the tumour with extreme precision. Tests have demonstrated that by using this targeted imaging method there is very little background noise, suggesting that this bioconjugate is accurate enough to detect cancerous cells at the single cell level [7].

In a study using a similar bioconjugate (docetaxel as opposed to doxorubicin), Farokhzad et al. (2006) measured the cytotoxic effectiveness of adding an aptamer targeting system to chemotherapeutic-nanoparticle bioconjugates. Using nude mice with a xenograft of cancerous human prostate cells, two approaches were compared to study aptamer effects in chemotherapy. One group of mice was administered a chemotherapeutic-nanoparticle including an aptamer to target specific cells. This procedure resulted in complete survival of all the mice, while 71% exhibited complete tumour reduction. In contrast, a second group of mice were administered chemotherapeutic nanoparticles without a conjugated aptamer guide. In this case, the mice exhibited complete tumour reduction in only 29%, with a survival rate of just 57%[7]. This experiment clearly demonstrated the potential benefits of using aptamers to specifically target diseased cells and the enhanced therapeutic efficacy of bioconjugate strategies. Not only is the aptamer included nanoparticle very effective, but it also appears to increase safety and decrease side effects. Regular chemotherapy is non-targeted and will affect all rapidly dividing cells in other organs and structures within the body. With a targeting system, only the desired tissues are subjected to chemotherapeutics, preventing unnecessary drug exposure[8].

BENEFITS OF USING APTAMERS

Aptamers are a promising new area of study and many of the patents involving SELEX have only recently expired. This has alleviated a major obstacle in aptamer utility for many innovative biomedical and environmental ventures. As a therapeutic, aptamers can easily replace antibodies as an inexpensive, customizable, and more effective option. Antibodies are time consuming to discover, difficult to generate, easily contaminated and have a relatively short half-life. Alternatively, aptamers are extremely inexpensive, easy to create quickly in large amounts, and are difficult to contaminate[9].

Due to the many possible applications, low toxicity, and extensive modification strategies, it is likely that aptamers will greatly influence the future of medicine. Research is being done on using aptamer-based therapeutics to treat “incurable” diseases such as lupus, cancer, and HIV, and has shown some very promising results. Aptamer research may also yield medications that can manage allergies and prevent migraines[9]. In addition to the biomedical applications listed above, aptamer technology is also being explored in the fields of environmental biodetection and food inspection[9].

CONCLUSION

With aptamers becoming a staple in biochemical research, the possible applications for this technology appear to be endless. However, one of
the most promising recent applications is their use as a component of bioconjugates for the treatment and imaging of prostate cancer. Prostate cancer, the most prevalent cancer among Canadian men, is being treated using strategies that result in numerous side effects. The method of using aptamers is more effective and may eliminate these undesired effects. Clinical trials are the next step for this treatment option, and if the trials yield results as positive as those in Farokhzad’s mice experiment, the use of aptamer-nanoparticle bioconjugates could become the standard of prostate cancer treatment.

With the prevalence of aptamers in biochemical research increasing, patent restrictions being lifted and discovery costs decreasing, aptamers may become a very popular and profitable treatment option for many diseases while concurrently increasing the quality of patient experiences.

**KEY WORDS** Aptamer; Bioconjugate; Quantum Dots; Prostate Gland; Doxorubicin

**ABBREVIATIONS**

DNA Deoxyribonucleic acid

PSMA Prostate specific membrane antigen

SELEX Systematic evolution of ligands by exponential enrichment

QD Quantum Dot

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**REFERENCES**


