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APPLICATIONS OF APTAMERS IN DRUG DELIVERY SYSTEM

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ABSTRACT

Aptamers, a kind of short single-stranded oligonucleic acids (DNA or RNA) or peptide molecules that can fold into secondary or tertiary structures, are well recognized as a potential tool for binding target molecules due to its high specificity and affinity. As tumor targeted ligands, aptamers have certain superiorities compared with antibodies, such as low immunogenicity, high binding specificity and so on. By the means of systematic evolution of ligands with exponential enrichment (SELEX) approach, selected aptamers showed high affinity to specific targets like proteins, peptides, and even the entire cells. In this review, we will give a brief overview of recent relevant research in the field of aptamers and their applications in cancer diagnostics and in drug delivery system.

KEYWORDS: aptamers, cancer, diagnostic, drug delivery system.

INTRODUCTION

When patients present with symptoms suggesting the likelihood of cancer, the next most important thing of doctor is how to diagnose. Molecular recognition of disease-specific markers, especially the recognition of proteins, or other molecules that differentiate between normal and abnormal cells, is a challenge in cancer diagnostics and treatment.[1] The discovery of new markers that are specific to particular cancers, as well as the development of molecular probes that bind to these markers, are two of the most critical issues in the field of cancer diagnostics today.

Aptamers,[2] originated from the beginning of the 1990s, are single-stranded DNA or RNA[3] with the ability to bind to non-nucleic acid target molecules, such as peptides, proteins, drugs,

or even whole cells, with high affinity and specificity.[4,5] As we all known, antibodies, peptides, small molecules and aptamers can all play the role of ligands. However, antibodies are associated with immune responses even though the high affinity for their targets. The enzymatic degradation of peptides resist their own *in vivo* application.[6] Furthermore, small molecules can be easily eliminated by kidney and display relatively lower targeting selectivity to cancer cells. Compared with the ligands above, the aptamers display a higher binding affinity to targets with dissociation constant (Kd) values in the nanomolar range.[7] Aptamers are consist of single-stranded DNA or RNA, so the immunogenicity and toxicity are very low. In addition, they can be storaged easily. With the help of systematic evolution of ligands with exponential enrichment (SELEX) approach, we can identify the aptamers sequences and once an aptamer sequence is identified, it can be synthesized with high purity and at relatively low cost.

Based on the recent relevant research, we will introduce the SELEX Process in detail, then, give a brief overview of aptamers and their applications in drug delivery system.

SELEX Process

Aptamers are generated by an interative *in vitro* evolution procedure named SELEX (systematic evolution of ligands by exponential enrichment). SELEX technology is an *in vitro* combinatorial chemistry process used to identify aptamers from large pools of diverse oligonucleotides, which can serve as specific ligands for a given target.

The whole SELEX process can be divided into several parts[8-11] (Fig.1). The starting point of the SELEX process is an establishment of a random DNA oligonucleotide library consisting of 10^{12}-10^{15} different sequences. Then the library was incubated with the target molecules (typically a recombinant protein). After incubation for a period time, the unbounded nucleic acids were partitioned from those bounded. Finally, we had to dissociate the nucleic acid-protein complexes, amplify the nucleic acid pool to generate a library of reduced complexity, then enrich in sequences that bind to the target. It is the end of the first round, the number of rounds is determined by both the type of library used and the specific enrichment achieved per selection cycle. After the final round, the PCR products are cloned into a vector and sequenced to allow for identification of the best binding sequences.

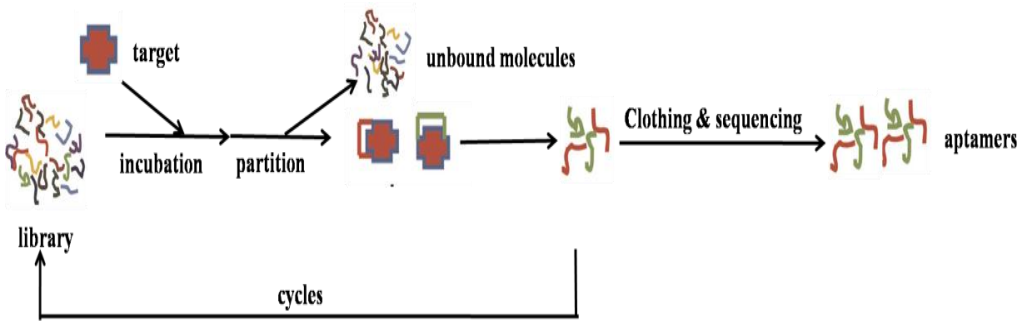

Fig 1: The process of SELEX.[12]

***In vitro* and *in vivo* cancer diagnostics**

To select the proper treatment or therapy for cancer disease, the quick and accurate diagnosis is very essential. Wherefore, a new kind of quick, accurate and easy detection method is urgently needed. Aptamers are very suitable for the purpose and will play an vital role in the development of accurate cancer detection. Many published research of cancer detection is based on the helpful molecules aptamers.

Colin D. Medley and his co-workers[13] developed a colorimetric assay for the direct detection of diseased cells. They connected the gold colloid nanoparticles (GNPs) and thiol-labeled DNA sequence (aptamers) to create a new kind of nanoparticles called ACGNPs for the sensitive detection of cancer cells. The result showed that the target cells incubated with ACGNPs exhibited a distinct color change, while for the control cells there was not any change in color. The assay also showed excellent sensitivity with the naked eye. This meant that the aptamer-conjugated gold nanoparticles could become a powerful tool for point of cancer diagnostics.

Alan K.H. Cheng et al.[14] reported a MUC1 aptamer-based, quantitative detection protocol for MUC1 using a three-component DNA hybridization system labeled with quantum dot (QD). In the absence of MUC1 peptides, strong fluorescence is observed after mixing the designed DNA strands (quencher, QD-labeled reporter, and the MUC1aptamer stem) while in the presence of MUC1 peptides, a decrease in fluorescence intensity is detected. The detection limit for MUC1 with this novel approach is in the nanomolar (nM) level, and a linear response can be established for the approximate range found in blood serum. The method offers the possibility of improvement in the early diagnosis of different types of epithelial cancers.

The history of aptamer being an introduced as imaging probes for *in vivo* studies can be traced back to 1997 when Charlton et al.[15] first used it. From then on, aptamer-nanoparticle conjugation forms the basis of a new chemical and biological strategy for *in vivo* imaging. The Do Won Hwang group[16] developed a cancer targeted imaging probe, using a multimodal nanoparticle conjugated with the AS1411 aptamer which has high affinity to nucleolus in protein over-expressed on the membrane of cancer cells. The MFR-AS1411nanoparticle monitored by fluorescent, radioisotope, and MRI modalities *in vivo* and *in vitro*. The scintigraphic imaging of nanoparticle in tumor-bearing nude mice showed that the nanoparticle was successfully targeted to cancer cells. Multifunctional imaging modality platforms based on aptamer-mediated nanotechnology represent the leading edge of molecular diagnostics and therapeutics on cancer and will affect our life in the near future.

Aptamer-Nanoparticles Systems

The past few years has witnessed the great advances in the synthesis and characterization of various nanomaterials, such as dots,[17,18] gold nanomaterials,[19] hydrogel[20] carbon nanotubes[21] liposome[22] and so on. The combination of aptamers with novel nanomaterials has led to a wide applications. Now, we will discuss how these nanomaterials have been used to functionalize aptamers for a variety of applications. These aptamer functionalized materials will lead to advances in cancer cell specific recognition and targeted drug delivery system.

Aptamer-drug nanoparticles

The aptamer-drug nanoparticles composed of therapeutic agents and aptamers via noncovalent or covalent conjugation are simple but efficient for targeted delivery.

The noncovalent conjugation do not need any chemical modification, which protects drugs and aptamers from losing their bioactivity. This kind of conjugation could typically achieve high drug-loading efficiency. Doxorubicin (Dox) (Fig.2) is a well-known anticancer drug shown efficacy against a range of neoplasms. Dox is known to intercalate within the DNA strand due to the presence of flat aromatic rings in this molecule.[23] Vaishali Bagalkot et al.[24] reported a novel strategy for the targeted delivery of Dox to cancer cells through the formation of an aptamer-Dox physical conjugate (Fig.3). The A10 2’-fluoropyrimidine RNA aptamer that binds to the prostate-specific membrane antigen (PSMA) with high affinity and specificity. They examined the this concept by using PSMA aptamer and Dox. The results showed that the cell uptake of the physical conjugate was obviously different between

LNCaP cells (a cell line in which the PSMA was expressing) and PC3 cells (a cell line in which the PSMA was not expressing). After incubation, the fluorescence intensity of Dox in LNCaP cells was higher than that in PC3 cells. Sorah Yoon et al.[25] made the P19 incorporated with gemcitabine and 5-fluorouracil (5-FU), or conjugated to monomethyl auristatinE (MMAE) and derivative of maytansine 1 (DM1). The cytotoxicity of P19-MMAE and P19-DM1 in normal cells and the control human breast cancer cell line MCF7 was minimal. These results meant that this approach may be useful in decreasing cytotoxic side effects in non-tumoral tissue.

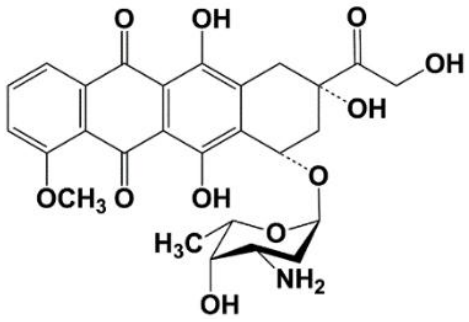

Fig 2: The structure of Doxorubicin.

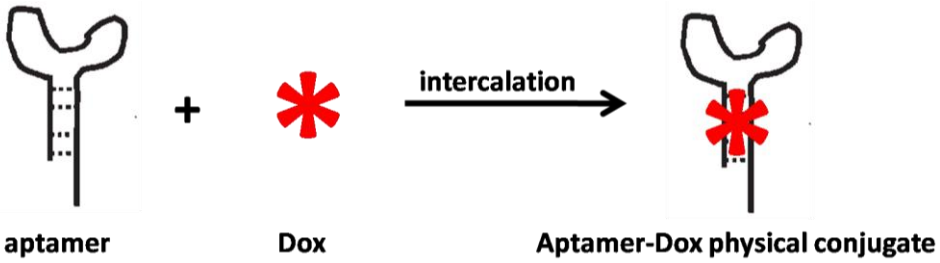

Fig 3: Physical-conjugate formation between an aptamer and Dox.[24]

Yu-Fen Huang et al.[26] covalently linked the antitumor agent doxorubicin (Dox) to the sgc8c aptamer which can recognize the protein tyrosinekinase 7 (PTK7), a transmembrane receptor highly expressed on CCRF-CEM cells[27] with high binding affinity. They assembled Dox into aptamer probe through a simple covalent conjugation method. The Sgc8c-Dox conjugates showed selective killing efficiency for different cancer cells.

Aptamer-Quantum Dots conjugates

Quantum dots (Qdots)[28,29] are a new kind of nanoparticle materials that have gained extensive investigation as potential drug delivery vehicles for its chemical stability, efficient and stable fluorescence signals, and superior biological probes. Qdots inherently possess numerous advantages over traditional fluorescent dyes such as increased photo stability,

higher brightness, and narrow fluorescence spectra. Aptamer conjugated Qdots have been performed since 2005[30] and later were widely used.

Ronak Savla et al.[31] reported a design and delivery of a tumor-targeted, pH-responsive quantum dot-mucin1aptamer-doxorubicin (QD-MUC1-DOX) conjugate for the chemotherapy of ovarian cancer. MUC1 aptamers (Fig.4) can recognize MUC1 which was overexpressed in all human epithelial cell adenocarcinomas[14,32-35] They connected MUC1 aptamer with Qdots. Dox was attached to Qdots via a pH-sensitive hydrazone bond. The data demonstrated a high potential of the proposed conjugate in treatment of multidrug resistant ovarian cancer.

Ling Zhu et al.[10] developed a novel fluorescent biosensor based on dopamine (DA) aptamer labeled carbon dots (aptamer-CDs) and nano-graphite (NG) for the determination of dopamine (DA). NG served as an energy acceptor and aptamer-CDs were in charge of energy donor and chemical recognition. Under the optimal conditions, the fluorescence intensity of aptamer-CDs increased linearly with the increase of DA concentration in the range of 0.10-5.00 nM and the limit of detection was 0.055 nM. This method was successfully applied to the determination of DA in human urine samples with satisfactory results.

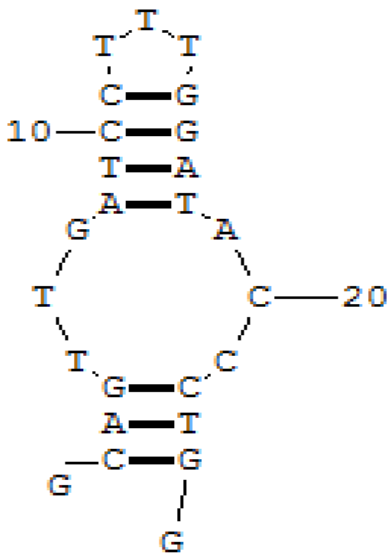

Fig 4: The structure of MUC1 aptamer.

Aptamer-AuNPs conjugates

Gold NPs (AuNPs) have gained much attention as drug delivery platforms because of their numerous advantageous properties. Aptamer-conjugated gold nanomaterials provide a powerful platform to facilitate targeted recognition, detection, and therapy.

Yun-Ling Luo et al.[36] devised a smart drug carrier, an aptamer/hairp in DNA-gold nanoparticle (apt/hp-Au NP) conjugate for targeted delivery of drugs. The sgc8c aptamer which possesses strong affinity for protein tyrosine kinase 7 (PTK7), abundantly expressed on the surface of CCRF-CEM (T-cell acute lymphoblastic leukemia) cells, was assembled onto the surface of Au NPs. The repeated sequence (CGATCG) within the hpDNA on the Au NP surface was used to load the anticancer drug doxorubicin (Dox). The apt/hp-Au NP conjugates' cell uptake of targeted cells was investigated by flow cytometry, which showed that the functionalized nanoparticles were selective for targeting of cancer cells. *In vitro* studies showed that conjugates can be used as carriers for targeted delivery of drugs.

Colin D. Medley et al.[37] used aptamer-conjugated gold nanoparticles to combine the selectivity and affinity of aptamers and the spectroscopic advantages of gold nanoparticles to allow for the sensitive detection of cancer cells. They developed a colorimetric assay for the direct detection of diseased cells with naked eyes. On the basis of this research article, aptamer-conjugated gold nanoparticles could become a powerful tool for point of care diagnostics.

Aptamer-CNT conjugates

Single-walled carbon nanotubes (SWNTs)[38] are one kind of carbon nanomaterials, they represent to be a promising tools for targeted drug delivery. Their unique properties including nano-needle structure enable them to easily cross the plasma membrane via endocytosis-independent pathway.

Marzieh Mohammadia et al.[39] developed a drug delivery system for breast cancer gene therapy based on a RNA aptamer (EpDT3) against Epithelial cell adhesion molecule (EpCAM), SWNTs and piperazine-PEI. This conjugates could efficiently increase DNA transfection. They successfully fabricated aptamer-conjugated nanoparticles for targeted delivery of siRNA to inhibit the expression of BCL9l in EpCAM positive cells through aptamer-mediated specific binding to the cell surface EpCAM.

Seyed Mohammad Taghdisi et al.[40] introduced sgc8c aptamer to complex between Dau (daunorubicin) and SWNT to enhance targeted delivery of Dau to acute lymphoblastic leukemia T-cells (Molt-4). The results showed that the Dau-aptamer-SWNTs was internalized effectively to Molt-4 cells(target cells), but not to U266 cells(control cells). The cytotoxic of complex was lower in U266 cells than Dau alone. Cytotoxicity of the complex

was efficiently and quickly reversed using antisense in Molt-4 cells. So Dau-aptamer-SWNTs complex is able to selectively target Molt-4 cells.

Aptamer-hydrogel conjugates

Hydrogels are polymeric networks with three-dimensional configuration that absorb large quantities of water or biological fluids, which can be formulated as macroscopic networks, or confined to smaller dimensions. It has been one of the most appealing polymeric materials used for preparing sustained-release systems that have a great impact on pharmaceutical development and regenerative medicine.[41] Hydrogel nanoparticles are outstanding drug delivery systems:[42] (1)The particle size and surface properties can be manipulated. (2)Controlled and sustained drug release at the target site, improving the therapeutic efficacy and reducing side effects. (3)Due to their tiny volume, they are ability to reach the smallest capillary vessels etc.

Zonghua Wang et al.[20] demonstrated the general design of a drug delivery system based on an aptamer cross-linked hydrogel. The AS1411 is a promoter which leads the hybridization of acrydite-modified oligonucleotides to form the hydrogels. But the presence of the target protein nucleolus reduces the cross-linking density by competitive target-aptamer binding, which makes the gel to dissolve. They encapsulated doxorubicin inside the gel during the formation of the hydrogel, then, the dox was released in the presence of nucleolus. In vitro experiment results confirm that the aptamer-functionalized hydrogels can be used as drug carriers in targeted therapy and other biotechnological applications.

CONCLUSION

In summary, aptamers are ligands with low immunogenicity, high binding specificity, which have attracted a growing interest as novel targeting molecules since their first discovery in 1990.[43, 44] In this review, we introduce the SELEX Process which is the beginning of the aptamers. In addition, we summarize the *in vitro* and *in vivo* cancer diagnostics as long as different kinds of aptamer-mediated drug delivery system. As discussed in this review, aptamers show good special targeted function to cancer cells but nearly no binding to normal cells as illustrated above. Thanks to the aptamer chemical versatility, many approaches have been developed to use them for nanoparticle functionalization. It will produce more exciting results in the targeted delivery system of tumor treatment and it could be reasonably expected that aptamer-targeted delivery system will have a bright future in few years.

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