A REVIEW ON APTAMERS IN GENE THERAPY AND THEIR APPLICATIONS

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ABSTRACT

Gene therapy has been initiated as long back from 1990s but is still in development. Research has been continuously going on in this field in order to cure genetic diseases by various techniques. One of these is aptamers. Aptamers are single stranded DNA or RNA molecules made by SELEX technology and have the ability of attaching to a variety of targets namely proteins, peptides, carbohydrates, toxins etc. They find application in skeletal disorders, biosensors, detection of viruses, delivery of drugs, various drug delivery systems, etc.

Keywords: Gene therapy, Cure, Aptamers, Skeletal, Biosensors, Viruses, Drugs

INTRODUCTION

Humans have been suffering from diseases since ancient times, which includes external ones like Scabies, Ringworm, Chickenpox and so forth as well as internal ones like Influenza, Pneumonia, Acquired Immuno Deficiency Syndrome (AIDS) etc. Though treating some of them is not easy but most challenging to cure are disorders known as genetic disorders, which arise due to mutation in genes or defective genes with which a person is born with due to passing on the genes from either of the parents coupled with many environmental factors which also play a role in pathogenesis of the disorder. These disorders although scarce, have an impact on millions of people all over the world. Examples include Lesch Nyhan Syndrome, Down’s syndrome, Cancer and Sickle cell anemia to name a few. In order to remedy such disorders, we require gene therapy. Gene therapy has been defined as introduction of nucleic acids into cells to treat, repair or replace and regulate defective genes to treat genetic diseases. The newly encoded nucleic acids will correct the faulty protein synthesis and the deficiency in the protein which was leading to the disease will be corrected [1]. There are many approaches towards this treatment like by employing antisense oligonucleotides, aptamers, liposomal gene delivery etc. Out of them, one such approach is by aptamers, which are defined as short single strand nucleic acid molecules, comprising of Deoxyribose Nucleic Acid (DNA) or Ribose Nucleic Acid (RNA) that attach to organic or inorganic molecules ranging from single atoms to an extensive variety of proteins. Aptamers are known by their high specificity to target molecule and binding power; dissociation constant of aptamer/target molecule complex comprising of repeated nanomolar units of RNA or DNA bases is high so that they do not separate easily [2]. Due to the advancements in Systematic Evolution of Ligands by Exponential Enrichment (SELEX), which is now a fundamental technique for the separation of aptamers, multiple aptamers can be instantly selected in vitro against many targets, ranging from compact biomolecules to proteins and even on a cellular level [3]. The adoption technique of aptamers SELEX is an in vitro method. Briefly, SELEX is based on repetitive cycles of binding, splitting and multiplication of nucleotides. The primitive step of traditional SELEX is to cultivate the sequence pool with the target (protein, nucleic acid, etc.). The sequence pool comprises of nucleic acid library containing $10^{14}$-$10^{15}$ revisions of random 30–100 nucleotides surrounded by persistent sequences at both ends. The usual region accommodates the sequences that will be evaluated for top specificity and affinity to the target. Second step is that the sequences that attach successfully to the target are retained, while unbound nucleotides are released from the cycle. The third step is to cleanse and multiply the fused sequences to form a novel sequence pool for the next cycle. This cyclic process is usually repeated 8-15 times before attaining the appropriate aptamer sequence pool which is needed to complete the process [4, 5].

Literature survey was done using accessible databases such as Google scholar, PubMed, Scopus to combine research articles. The objective of this review is to provide all the literature on aptamers in the last 10 years followed by their applications.

Aptamers

The structure of aptamer used therapeutically is given below as shown in fig. 1.

Fig 1: Structure of aptamer pegaptanib (Macugen), the foremost nucleic acid aptamer to be given permission by the US FDA for clinical use [6]
Adapted by permission from the ATD Bio (Chemical industry in types of cargos at specific targets by conformational switching. This transformation failed to fuse with the aptamer [16]. DNA aptamers human body [15]. A fluorescent-conjugated DNA aptamer was cells with the hazard of malignant metamorphosis. As a , HPV is a intermolecular hybridization with complementary nucleic acid s. conformational change is induced by a molecular effector or by defined the target of constructed aptamer as a glycosylated HA phi29 packaging RNA (pRNA) molecule s were employed to unite it rapport and specificity [7]. The characteristics  of bacterial virus need any animals or specific cell lines; thus they can be pushed up viral diagnostics with aptamers have been made possible by expanding the benefits of aptamers. Another salient area of applications is the detection of chronic infections caused by viruses or even allied strains of the same influenza subtype. It should be noted that P30-10-16 latches to the target molecule (H3N2 of virus A) with more than 15-fold elevated affinity, as compared to standard aptamer HA monoclonal antibody. Just now, aptamers were manufactured which are able to detect influenza virus type A: HI1 and H3N2, along with avian virus, H5N1. It is still indispensable to make simple and widely available methods in order to further expand the benefits of aptamers. Another salient area of applications of aptamers is the detection of chronic infections caused by viruses of hepatitis B and C [11]. Aptamer specific for HIV glycoprotein was unearthed, which brought into light a novel diagnostic test—Enzyme-Linked Apto-Sorbent Assay (ELASA)—which permitted for qualitative analysis, in addition to quantitative analysis of virus particles in the assessed samples [12]. An important impact for expansion in viral diagnostics with aptamers has been made possible by constructing gold microelectrodes with impedimetric properties in order to decide between biologically active from inactive form of the virus and used heat-inactivated vaccine as a prototype. Using fixed set of DNA aptamers which were exposed, the presence of the viable virus form could be identified since the probability of error was lowered significantly [13]. Similar study was taken up, in which it defined the target of constructed aptamer as a glycosylated HA molecule on infected cells. It is important to blossom the line of fast, quick responding, and economically diagnostic tools, to pinpoint the type of virus infection, as well as detection of dysplastic endometrial cells with the hazard of malignant metamorphosis. As a, HPV is a prospective target for aptamers [14]. An experimental done by making use of in vitro selection to obtain the RNA aptamer that tethered to viral E7 oncoprotein. Being a section of the biosensor it could equip a powerful diagnostic tool which helps to detect virus within the human body [15]. A fluorescent-conjugated DNA aptamer was developed that has the power to bind surface determinants of the normal cervical epithelium. The cells that started malignant transformation failed to fuse with the aptamer [16]. DNA aptamers were constructed, which were targeted as antagonists towards the pathogens of the arbovirus family, including a variety of viruses, such as Chikungunya, Crimean-Congo Hemorrhagic Fever, Dengue, West Nile and Tick-Borne Encephalitis. They got sequences with high precision and rapport for both the clean recombinant viral vaccines and whole infectious viruses. The E12 virus-like particles (VLPs), chosen sequences in forecasted diagnostic methods, i.e., lateral flow chromatographic test strip and fluorescent aptamer-magnetic bead sandwich assay. They also brought forward the aptamers to be serviced for plant immunity and antiviral prevention because of the shallow immunogenicity. This technique could be bright in any dangerous infection with shortfall of well-organized therapeutic replacement. The application of biosensors loaded with the aptamers or differing research techniques by adopting these molecules permits for sensing of both: early (genetic material, viral proteins) and late (host own antibodies) infection markers [17]. There is a high probability of infection and astonishing frequency of mutations, leading to a cyclical emergence of novel viral strains with epidemic or even pandemic danger as a result focused attention was given to influenza diagnosis. It is still necessary to generate simple and useful techniques in order to increase the benefits of aptamers [18]. To weaken HIV-associated cardiomyopathy an anti-glycoprotein120 aptamer called as UCLA1 was established. It directly fuses to HIV-1 thereby destroying the virus, thus granting cardiomyocytes from cell death and indirectly halted infection of monocyte-derived macrophages in the heart [19]. Diagnostic works with aptamer-based biosensors performed on natural or clinical-based samples

The efficacy of aptasensors in the recognition of viruses was also evaluated in clinical samples. An experiment was undertaken to merge aptamers with biosensor technology, known as Surface Plasmon Resonance (SPR). This model incorporates a fragment coated with both gold coating and Streptavidin as the drug DNA aptamers launched against avian influenza H5N1 have been fixed to its surface, since they were reconstructed by biotinization; the material altered in the study was saliva collected from the poultry. The method has absence of labeling procedures and enables quick (1.5 h) detection [20]. In another study twin aptamers called as IF10 and IF22 were constructed, became attached at different sites of (1.5 h) detection [20]. In another study twin aptamers called as IF10 and IF22 were constructed, became attached at different sites of (1.5 h) detection [20].

Pros and cons of aptamer-based tests in comparison to other diagnostics methods

The ongoing diagnostic methods for detection of viral infections are mostly ELISA or molecular biology tests, which are routinely employed [20, 23, 11, 14]. Repeatedly used ELISA is a multi-stage method and is less efficient due to the fact of relatively shallow sensitivity as well as high incidences of false-positive results [20, 23, 11]. As a substitute regularly used or even investigational diagnostic molecular tests permit for quickest detection of the new genetic material, without standing by for the immune reaction of the host. Furthermore, the supremacy of molecular assays is their very high sensitivity: they have the ability to detect virus present in a trace amount, and/or early viral transcripts right away after theogenesis of infection [25, 26]. But these have their shortcomings such as not economically feasible,
Applications of aptamers

Aptamers in bio imaging

One application of aptamers is in bio-imaging, where employing an aptamer that is fused to a fluorophore, a quantum dot (QD) or other materials such as gadolinium which is functional for magnetic resonance imaging (MRI). Using aptamers as imaging agents has the superiority of being non-toxic due to the fact oligonucleotide moieties exist in the human body. Results were published of glioma cell line (C6) cell imaging using a dye called Cyamine 3.0(cy3) which was labeled onto AS1411 aptamer, which had undergone chemical modification of 5-(N-benzylcarboxyamide)-2-deoxyuridine(5'-BzdU) on a thymidine base. The AS1411 aptamer is particular for the nucleolin trans-membrane protein present in cancer cells. Specifically, this group enhanced the aptamer’s binding power to its target via a chemical modification. The cell imaging of the altered Cy3-labeled AS1411 aptamer was more systematic for the C3 cells as compared to the original Cy3-labeled AS1411 aptamer [33]. Assembly of QD-A10 and DUP-1 aptamer complex was done, which is particular for Prostate-specific membrane antigen [PSMA (+)] and [PSMA (-)] prostate cancer cells (LNCaP and PC3), was demonstrated by imaging to attach only to prostate cancer cells instead of normal (PNT2) or other cancer cells [34].

Aptamers in delivery of anthracycline drugs and imaging

The majority of theranostic studies with aptamers involve the co-delivery of imaging agents in addition to anthracycline drugs (predominantly Doxorubicin). These drugs deal with DNA by intercalation, halting replication, and disrupting gene expression. The first way to administer the drugs was to entrap them in different nanoparticles that were further labeled for recognition by an auxiliary (CGA)7 repeat that was inter-mixed to a prostate tumors was developed. The extension the sequence of the membrane antigen (PSMA), which is overexpressed on several prostate cancer cells (LNCaP and PC3), was achieved by reversing the fluorophore. It was also armed with Doxorubicin, as the sensitive hydrazine bond. These bonds do not break down in blood at a neutral as well as slightly basic pH but undergo fast hydrolysis in the acidic surrounding of endosomes. As a result it should free the drugs only in cancer cells that have nucleolin because they should incorporate the nanoparticles via the aptamer. In vivo fluorescence imaging showed that the quantum dots amassed more in subcutaneous xenografts when they were joined to the aptamer. Furthermore, the therapeutic response of these nanoparticles were enhanced marginally by the aptamer in vitro, but was not assessed in vivo due to some issues [38]. Doxorubicin was also included to the surface of shining gold nano-clusters which was further bonded with two specific targeting moieties: the anti-nucleolin aptamer and a cyclic Arg-Gly-Asp (cRGD) peptide, which joins to αvβ3 and αvβ5 integrins, which are overexpressed within tumor endothelial cells. In vivo fluorescence imaging showed considerable higher tumor targeting when the nanoparticles were joined with the dual targeting agents than when they were alone and somewhat greater tumor-targeting than nanoparticles bonded with the only cRGD. Intravenous injection of the nanoparticles bonded with the dual targeting agents every 48 h generated higher retardation of tumor growth than non-bonded nanoparticles and also on Doxorubicin alone [39]. An aptamer that bonds to the prostate-specific membrane antigen (PSMA), which is overexpressed on several prostate tumors was developed. The extension the sequence of the aptamer by an auxiliary (CGA)7 repeat that was inter-mixed to a sequence conjugated to the targeted particle. This loading strategy was performed. This dual strand matching was used to conjugate the aptamer as well as for the entrapment of Doxorubicin, which is known to preferably attach to following cytosine and guanine base pairs. The biodistribution of this complex was then checked by MRI in nude mice showing subcutaneous tumors. Following an hour after administration the nanoparticles bonded with the aptamer showed ten-fold greater tumor-targeting than those joined with a scrambled control sequence [40]. A similar loading strategy employed which involved entrapment of a MUC1 aptamer that was joined to SPIONs, with Eribulin. MIRI demonstrated higher tumor targeting of the nanoparticles conjugated with the aptamer than unconjugated nanoparticles, similarly to the previous study. Furthermore, they also demonstrated greater retardation of tumor growth [41]. The ability of Doxorubicin to intercalate into DNA was also exploited to construct an intelligent split aptamer-based activatable theranostic probe. They employed the Sgc8c aptamer that deals with a cancer-related membrane PTK-7. The Sgc8c aptamer was broken into two parts and joined via a short DNA linker labeled with a fluorophore. The central part of this linker was then inter-mixed with a complementary short DNA strand to which was linked to a quencher that diminishes the fluorescence emission of the fluorophore. It was also armed with Doxorubicin, as the double helix region linker/short DNA strand was guanine and cytosine rich. The model was designed to be pulled apart when the

| Virus   | Method    | Detection limit | Advantages                  | Disadvantages                  | References          |
|---------|-----------|-----------------|-----------------------------|-------------------------------|---------------------|
| Influenza | qRT-PCR   | 0.0256 HAU/ml   | Specificity                 | Expensive                     | Dhumpa R et al. 2011 [27]. |
|         | RT-PCR    | 1.785 IU/ml     |                          | Complicated, highly skilled stuff | Sidoti F et al. 2010 [28]. |
| HBV     | ELISA     | 0.5 pg/ml       |                          | Expensive                     | Yang J et al. 2010 [29]. |
| HIV     | ELISA     | 0.9–1.2 IU/ml   |                          | Expensive                     | Mühlbacher A et al. 2013 [31]. |
|         | qPCR      | 3.21–11.60 IU/ml| Sensitivity                | Complicated, highly skilled stuff | Saune K et al. 2013 [32]. |

Table 1: Comparison of traditionally used diagnostic techniques in the detection of HIV, HBV and influenza

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Aptamers in delivery of taxane drugs and imaging

An overview of aptamers employed in various drug delivery systems, including cancer and other diseases, is shown in fig. 2.

Fig. 2: An overview of various aptameric systems used in drug delivery [43]. A] Entrapment of aptamer drug complex in liposome, B] Aptamer antibody conjugate for targeting selective genes, C] Drug streptavidin serving as a base for connecting two aptamers and two siRNA molecules, D] Drug 5-flourouracil shown in red connected to the aptamer oligonucleotide backbone via a photocleaveable linker which is shown in blue, E] Chemical structures of Tobramycin (red) and Doxycycline (blue)

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Several theranostic studies have been conducted with aptamers for the co-delivery of imaging agents and taxane drugs, such as Paclitaxel (Taxol) and Docetaxel (Taxotere), which are commonly employed as chemotherapy agents. The main mechanism of action of these drugs is to wreak havoc upon the microtubule duty that is pivotal for cell division. Opposite to anthracyclines, which are water-insoluble, taxanes are water-insoluble. Thus, the theranostics nanoparticles were designed for those drugs which are not alike. A method was established using a nano-precipitation process with an acetone/water system to build nanoparticles of cholic acid-derived with a star-shaped block copolymer comprising of polyactic glycolic acid (TPGS) and Vitamin E armed with Docetaxel and a near-infrared fluorescent dye. The surface of these nanoparticles was moreover linked with the anti-nucleolin aptamer. The constructed nanoparticles were checked in two mouse models wherein the mice obtained subcutaneous tumor xenografts from human breast cancer cell lines. The nanoparticles linked with the aptamer showed four-fold higher tumor-targeting 24 h following intravenous injection than non-bonded nanoparticles or free dye by fluorescence imaging. Furthermore, they also illustrated higher inhibition of tumor growth [44]. The anti-nucleolin aptamer was also exploited again to target poly (L-c-glutamyl-glutamine) nano-conjugates armed with Paclitaxel as well as a fluorescent dye. In contrast to most of the studies described above, these nanoparticles were not checked in subcutaneous models but in nude mice that institute orthotopic brain tumors from an intracranial injection of human U87 MG glioblastoma cells. Fluorescence imaging showed two-fold higher tumor targeting by the nanoparticles linked with the aptamer than lone nanoparticles 24 h following intravenous injection. Additionally, when the nanoparticles were administered every three days, the median life span of mice that obtained the nanoparticles (52 d) was substantially prolonged as compared to those treated with unconjugated nanoparticles (47 d), free Taxol (40 d), or a control saline solution (36 d) [45]. Not so different conclusions were seen in an alternative orthotopic brain tumor model. The also similarly employed the anti-nucleolin aptamer joined to the surface of poly (ethylene glycol)-poly (L-c-caprolactone) nanoparticles armed with Docetaxel and fluorescence dye. The surface of the nanoparticle was also joined with a peptide earlier protected by phage display to travel across the Blood-Brain Barrier (BBB). Fluorescence imaging illustrated that this peptide improved brain ingestion of the nanoparticles as well as the addition of the aptamer, further enhanced ingestion of the nanoparticles by the tumor [46]. The same nanoparticle was used, which in turn attained the same result using an alternative aptamer chosen to recognize glioblastoma cancer cells in the absence of the peptide to travel across the BBB. This may be accountable to the truth that BBB is imaginarily hampered throughout the development of the brain tumor [47].

Aptamers in miscellaneous applications

Hydrogels are inter-linked polymers comprising of a 3 Dimensional (3D) network. It is true that hydrophilic group is often present in chemical structures of many compounds, including hydrogels containing groups such as carboxylic acid (COOH), imine (NH), hydroxyl (OH), sulphonic acid (SO3H) and carbboxamide (CONH), hydrogels also possess the capability to hold and absorb a fixed amount of water which leads to their swelling when they come in contact with water thus forming the 3D network [48]. Aptamer-derived hydrogels can be scheduled to deliver many and multiple therapeutics when required through particular nucleic acid identification and complementary inter-mixing process. Recently a nucleic acid-based affinity hydrogel system for controlled protein release was assembled. Two variable nucleic acid aptamers, as well as complementary sequences, were employed to control the delivery speed of Vascular Endothelial Growth Factor (VEGF) and platelet-derived growth factor having two B subunits (BB) in vitro [49–51]. A novel aptamer-functionalized hydrogel for controlled cell capture and liberation was fabricated. The customizable hydrogel comprises of a chief Complementary Sequence (CS), which is an aptamer sequence and a subsidiary CS. Firstly, the main CS was linked to the hydrogel resting on a solid support surface. The aptamer was inter-mixed with the key CS and can only bind bond with cells via polyvalent aptamer-protein interactions. When the secondary CS was applied to trigger the hydrogel, the aptamer broken apart from the main CS and then inter-mixed with the subsidiary CS. Therefore, polyvalent interactions between the cells and the hydrogel were diminished, resulting in a safe release of the cells from the solid surface [52]. An aptamer-derived hydrogel as a synthetic Extracellular Matrix (ECM) for cell fixing without compromising cell viability was fabricated [53].

structure of the aptamer encounters metamorphosis during its interaction with PTK-7, delivering the Doxorubicin and activating the radiation of a fluorescent signal. Both therapy as well as imaging, were tested in vitro. The checking of the construct in vivo by fluorescence imaging in nude mice bearing subcutaneous tumors was also carried out. The tumor demonstrated high fluorescence, just 15 min post-injection, but the clinical response was not evaluated [42].

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Aptamers for skeletal disease therapy in preclinical studies

Stem-cell-based therapies are one of the most innovative and rewarding fields of making advancements in the treatment of diseases in humans [54]. Stem cells are those cells which are in their premature state and have not yet been differentiated to handle a specific task. These cells are also immortal owing to their inability to age over time coupled with the ability to keep multiplying forever and such cells have the power to transform to variable cell types like skin, liver, kidney, heart, nerve cells and so forth depending upon the type of organ in which they are introduced [55]. The cell count of bone marrow mesenchymal stem cells (BMSCs) is diminished through aging while enhanced through adipocyte polarity. It is already demonstrated that miR-188 level is much greater in BMSCs in old as compared to young mice and human. Animals deficient in miR-188 can be safeguarded from age-related bone loss and fat collection in bone marrow. An aptamer that only recognizes BMSCs is constructed and linked with miR-188 to form a nano complex was constructed. This targeted transported nano-complex could enhance bone generation and lessen fat collection in bone marrow with more clinical results in aged mice, showing a promising approach for age-related bone loss cure [56]. Another preliminary study gave birth to a windowed aptamer targeted against human jaw periosteal cells (JPCs) is constructed for tissue engineering in oral and maxillofacial surgery. This aptamer has great power to bind to human osteogenically generated JPCs and BMSCs from bone marrow while it shows an absence of binding to any other cell lines or undifferentiated JPCs or JPCs born from alternative sources. It can be used to cleanse osteogenic parent cells from undifferentiated JPCs or stem cells from another sources. The mineralization ability is greater in the positive aptamer group, which is an encouraging method for tissue engineering [57].

CONCLUSION

In order to materialize a proper therapy for genetic diseases it is necessary to understand what those diseases are and how they can be treated using methods such as aptamers. The literature work done in aptamers and subsequently its applications were discussed. This article will definitely help all researchers, students, as well as academicians who are working in this field. Even now advancements are currently going on in this field and in the future further developments will ensure that genetic diseases are cured successfully and people who are suffering from this disease will lead normal lives.

ACKNOWLEDGMENT

The authors are thankful towards Mrs. Faiza Shaikh for providing guidance during the preparation of this manuscript.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have taken part in the design and drafting the article and revising it critically for important intellectual content as well as approval of the final version.

CONFLICT OF INTERESTS

Declared none

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