Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(5):196-203

Review Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The Applications of Aptamers in the Field Of Therapeutics

Sila Miso, Rutabana Aude, M. Rajasulochana, R. Muthulakshmi and Jasmine R.*

Department of Biotechnology and Bioinformatics, Bishop Heber College, Trichy-17, Tamil Nadu, India

---

ABSTRACT

Aptamers are single stranded nucleic acid which can bind directly to various protein structures by specific 3-dimensional structure and results in high affinity attachment to targeted site. They are found more advantageous than monoclonal antibodies which is drawn back due to immune rejection system but also instead of hybridization molecular strategies it goes similar with antigen and antibody binding mechanism and also similar in respect of binding (high specific) in picomolar or nanomolar range. Aptamers is an effective antagonist against protein functions and other biomolecules such as lipids, carbohydrate, other organic compounds and even whole cells. Thermal stability, recovery of native conformation after denaturation, easy and cost effective in production, feasibility of chemical modification with requirement, nuclease resistance; less immunogenic, highly binding specificity and affinity are some of positive aspects preferred in therapeutic and diagnostic requirement agenda. The applications of aptamer is wide and prospective, keeping in mind all the properties and affinity towards various biomolecular substance, the complete significance of aptamer is still long way to reach the common people in affordable price.

Key words: Aptamers, application of aptamers, selex, therapeutics and diagnosis

---

INTRODUCTION

"Aptamer" word derived from Latin "aptus" means "to fit". Unlike antisense compound In every disease or disorder be it genetic or somatic there are certain possibilities that is either irregular protein functioning, over production of protein or there is no production which leads to term call disease or disorder also depends on factor that may be genetics, viral or other microbial. Therefore Correction of irregular gene expression either due to over expression or no expression is needed. In this review the important criteria of aptamers and its recent advancement are cited, Limitation of every technology and findings after some extent, transforms into disadvantage. Many disease and disorders is still costly against human life such as cancer, HIV, neurological disorders etc which conclude, the aptamer a better choice when compared to several other technologies and their limitations and also the possibilities of aptamer transforming into a novel approach in the field of therapeutic aspects.

Aptamer is a short length oligonucleotide which is in general a designed blue print developed against target ensuring the safety of it from the target defense mechanism designed using chemical modification. The presence of various nucleases in the female genital tract that easily degrade nucleic acid aptamer is overcome by chemical introduction of 2'-O-Me modification in the purine nnucleotide or phosphorothioate linkages. Zinc ions are also found effective against nucleases due to sensitive behavior of nucleases to zinc ions [1]. Similar modification is done for treating sexually transmitted viruses, such as HIV-1 and HPV that is targeted by intravaginal application of a microbicide or cream that contains the neutralizing aptamers [2]. Aptamers are ssDNA, RNA oligonucleotides or peptide. It forms

various form of 3-dimensional structure to target protein, generated by process called SELEX. High affinity and specificity towards the targeted protein makes an aptamers unique and special. When compare to RNA aptamers DNA aptamers were found more stable in behavior, due to the absence of 2-OH group. Instead of difference in stability both have highly binding affinity for the target molecule [3].

SELEX FOR PRODUCTION OF APTAMERS REVIEW

SELEX (Systematic evolution of ligands by exponential enrichment) helps in isolation of aptamers from randomized pools of sample. It was first developed by Turk and Gold, which is an extremely powerful purification method, which helps in isolating even rare binding with frequency of 1 in 10^{11} to 1 in $10^{13}$from a large combinatorial library (4) SELEX mainly consist of 3 general steps, first the incubation of initial pool of nucleic acid with the target, Followed by separation of the target bound and unbound nucleic acid molecules, Finally amplification and regeneration of the bound nucleic acids. In production of DNA aptamer RNA aptamer do not require extra steps such as DNA aptamer production which requires the conversion of the ssDNA from dsDNA. In SELEX technology Proteomic knowledge is used to obtain high affinity ligand or probes that specifically recognize protein targets in assays, Versatility of aptamer and its tremendous potential in using as affinity molecules ease in production. The regeneration and stability is due to the chemical properties of nucleic acids versus amino acids. Aptamers are characterized by using surface plasmon resonance which is found to be useful in enzyme-linked assays, western blots and affinity purification. The short and single-stranded DNA or RNA aptamer sequences are selected in vitro based on affinity for a target molecule (5).

CHEMISTRY BEHIND APTAMER SPECIFICITY

"Chemical antibodies" is a term used for aptamer. The principle behind the specificity and affinity of aptamer is of chemical modification which helps in enhancing the targeted requirements. Oligonucleotide terminal caps, phosphorothioate internucleotides linkages, 2′-fluoro-ribose and 2′-*O*-methyl ribose are some of useful modifications done in designing. Functions of artificially design aptamer are diverse, which include identification of enzyme functions, protein bio distribution, tracking specific biomarkers and specific drugs delivery. Even with limited number of base pair contentment oligonucleotides hold complete functional groups that brings similarity with natural base pairs in reaction and function.

ROLE OF CONJUGATES AND LINKER IN APTAMER SPECIFICITY

The certain factors given strong attention by researchers while designing aptamer is conjugates and linkers. The efficient task of Conjugates contribute maximum efficacy of aptamer and bring down toxicity in target [6]. The chemically synthesized aptamer accept target introduction of non nucleotide linkers, the rate of ligand binding is always based on linker size, the chemical composition, increase and decrease of linker molecules and its effects on ligand bindings. A systematic study [7] reported, the effect of linker size and its chemical composition toward ligand binding to a surface-immobilized aptamer, which was measured with surface plasmon resonance, where thrombin was used as the model system, resulted in increase In thymidine (T) number, also the units in the linker increases from 0 to 20 in four separate increments (T0, T5, T10, T20), the surface density of the aptamer decreased linearly from approximately 25 to 12 pmol.cm-2 [8]. The decrease in aptamer surface density Occurred due to the increased size of the linker molecules. But in addition, thrombin binding capacity was shown to increase as the linker length increased from 0 to 5 thymidine nucleotides. The initial increase was due to increased in access of thrombin to the aptamer as the aptamer was moved away from the surface. Mostly Linkers don't interfere terminal nucleotides specifically instead it spatially extends the receptor from the surface in order to increase accessibility with the help of solution ligand it also removes non-specific Adsorption [9]. In aptamer the attachment of the linker at the 3'versus the 5-end result in increase aptamer surface density. Incorporation of a hexa (ethylene glycol) moiety into the linker did not affect the surface density, but increased the binding affinity. The effective binding specificity and affinity is absolute in aptamer selection, without which the assessment of prolonged effect is indistinguishable [10]. It is solvent exposed; ethylene glycol that gives high conformational flexibility and also acquires water solubility. The reason behind widely acceptance of aptamers in designing therapeutics and other application is known sequence of DNA or RNA which makes things easier to used against targeted protein and other targeted molecules [11].

DIFFERENT TYPES OF APTAMER

The chemical modification is generally taken care while in SELEX method, to discourage the susceptible nature of nucleic acid against nuclease and its active participation in nucleic acid degradation initiated by renal filtration, serum nucleases, liver functions, and various other tissues [12]. Aptamers being ssDNA, ssRNA, oligonucleotides or peptide encourage various forms of 3-dimensional structure against target molecules [13] When compare to ssRNA

and ssDNA aptamer, DNA aptamer is found stable than RNA due to the absence of 2-OH [14] but the fact that existed behind production of DNA aptamer is conversion of dsDNA to ssDNA, which needs separate attentive techniques, such as magnetic separation [15] nuclease digestion, [16] PCR techniques and denaturation methods [17]. RNA aptamers is easy in terms of synthesizing large quantities in given controlled environment it has a good property in achieving defined structure and stoichiometry [18]. Chemically modification can make progress in stability used in blood stream and resistance to RNAase shearing [19]. Potential nature of RNA are ribozymes, short hair pin RNA(shRNA), siRNA, miRNA, antisense oligonucleotides(AS OGNs) and RNA aptamers which has been extensively investigated topic therapeutic research [20]. Oligonucleotide aptamers is among the diverse types of nucleotide which is the choice of subject due to various prospective reasons such as due to alteration of nucleic acid artificially which draw them potential towards targeted protein, where short length with only functionally important length is retained.

APTAMER, A RIVAL AGAINST ANTIBODY

Though antibody holds prevailing effects and efforts in therapeutic world it also inherited flaws of biological system, countable draw backs are, rely on animals which draws them near to ethical issues, microbial contamination in product is an another aspect which needs great attentions similarly it is generally immunogenic that require another strategies to be implemented, production in commercial height is held back due to use of biological system, require cellular biochemistry manipulation and its acceptance as well as perfect stability [21]. Aptamer is similar to antibody, designed to bind multiple binding sites and targets, the crucial advantage of it is, it recover easily its native 3D conformation after denaturation even at 95°C owing to its own intrinsic properties where as antibody lose its activity along with conformations at high temperature. Aptamers get easily bind to target after reannealing and acquire no possibility of contamination from microbial population, in terms of synthesis and stability due to chemically driven it can be modified in accordance of requirement moreover the affinities of aptamers with target protein is much stronger than one can find in fab fragments and their target antigen [22]. The SELEX dependent aptamer encourage very less immune rejection which make it a better choice.

APTAMERS BASED THERAPY AGAINST VIRAL PROTEINS

The obligate parasite virus and its ambitious behavior of overtaking gene expression mechanism in living body for their own product generation, is quite harmless when it's toxic responsible protein and other biomolecule is stopped from functioning. The antisense therapy, hybridization technique, ribozyme therapy mainly target against translation and transcription where as aptamer is against all biomolecules responsible, in case of Retroviruses and many other viruses, they develops a small size, structured RNA sequences to recruit viral and host cell protein to perform essential functions in viral replication. It was in the year 1992, Tuerk and Gold observed 1st aptamers against HIV isolated from RNA pool. HIV AIDS also termed as non curative disease except the slowed down of progressing effect up to a limited extends using combination Antiretroviral therapy (cART) there is no permanent cure and more over due to high cost of cART therapy and sometimes extreme side effects made this slow down process less effective. After the findings of tuerk and gold the first aptamers it is been observed that many such aptamers can be designed against proteins and molecules responsible for invasion of HIVAIDS viruses in human body which include Reverse transcriptase (RT), TAR, Rev Tat, integrase, nucleocapsid, Gag, and gp120, whose inhibition has perfectly proved success in inhibition of viral replication and its effect [23]. HIV-1, HCV, HBV, SCoV, Rabies virus, HPV, HSV and influenza virus are some of human viruses, where the extensive use of aptamer is currently in progress with particular focus on clinical development of aptamers.

TAR AND RRE, AN IMPORTANT RNA SEQUENCE

TAR and RRE is an important RNA sequence which is the blue print of viral regulator protein TAT and REV in HIV AIDS virus, which is employed by virus in human host in order to induce gene expression. This overtaking capability of virus was first identified by employing a short transcript corresponding to the TAR RNA sequence [24]. To stop the viral gene expression, the decoy RNA is made to compete against virus encoded TAR sequence for binding to TAT results in Inhibition of HIV RNA transcription. Decoy RNA is isolated from real tar RNA sequence present in the HIV viral RNA. High Expression of tar decoys in T-lymphocytes and $CD4^+$ T-cell lines is observed which act persistently resistant towards HIV replication. Above experiment signifies that short RNA decoys are not only against antiviral, it can be against any specific proteins responsible. The followed principle is currently translated to the clinic, and the safety, feasibility and efficacy is evaluated in pilot clinical trials following retroviral mediated gene transfer into CD34 cells from the bone marrow of hiv-1 infected pediatric patient [25] to block all the major enzymatic steps catalyzed by RT and to inhibit HIV replication in cell culture [26] Thus aptamers that bind RT are promising reagents for use as therapeutics, diagnostics and research tools.

APTAMERS IN CANCER CELL DIAGNOSTICS AND TREATMENT

Cancer represents a group of diseases, which almost affect any part of the body. It is among the leading cause of death worldwide, affecting around 13% of the population. Cancer is a major public health problem and an economic burden. The goal is to detect and treat the disease in its early stage, in order to achieve a better outcome. However, the main problem is the lack of sensitive and specific methods for discovering cancer in early stages, due to the small amount of circulating cancer cells and low expression of biomarkers or specifically molecular markers. Chemotherapy the main supplementary therapy is a toxic for healthy cells. However, this is where aptamers can be applied, due to their ability to detect cell-specific markers within complex systems that are devoid of specified target molecules. Moreover, aptamers, which are highly specific for cancer cells, can be conjugated with therapeutic agents or small interfering RNA (siRNA). Aptamers can also be used directly, as medicinal substances, because of their toxic effect on certain target cells. They can be applied as regulators of the intrinsic intracellular pathway, due to their inhibitory effect on oncogens, and thus inhibit tumor growth and invasion [27]. Cancer even after decade still is the leading causes of death worldwide. Cancer types which remain the most dangerous are lung (1 370 0000 deaths), stomach (736 000 deaths), liver (695 000 deaths), colorectal (608 000 deaths), breast (458 000 deaths) and cervical cancer (275,000 deaths). The main challenge in cancer medicine is associated with early detection and specific treatment of cancer. The critical point is especially low expression of biomarkers in early stages and toxicity of chemotherapy. Oligonucleotides which are capable of binding to molecules other than nucleic acids are named aptamers. Which are single stranded DNA or RNA oligonucleotides (12-30 bases), used against cancer cells and cancer biomarkers and also enrich rare cancer cells with biotechnological, nanotechnological and analytical methods. Furthermore, they can be used as therapeutic agents [28].

APTAMER ABILITY TO RECOGNIZE ONCOGENS

Therapeutic features of aptamers seem to be linked with their ability to recognize tumor markers or oncogens, the potential to couple drugs directly or to pack it into particles modified with aptamers. Regarding the literature, aptamers in medicinal is generally distinguished into: aptamers as intracellular delivery vehicles – intramers [29] Aptamer-directed drug conjugation [30] Liposome conjugates for target drug delivery [31] Aptamer-micelle conjugates for target drug delivery [32] Aptamer-protein conjugates [33] Aptamer radionuclide conjugates and aptamer-nanostructure conjugates [34] Expression of protein over production, altered transcription factors, growth factors, receptors, intracellular mediators and mutation in tyrosine kinase activity act as oncogenes and cause cellular transformation. Increase in drug resistance result in failure of cancer therapy requires Targeted drug delivery within solid tumors that helps in complete drug penetration and durable retention.

CELL-SELEX FOR PRODUCTION OF CANCER SPECIFIC APTAMER

SELEX, the cell-SELEX or the brasil technology are the method of selecting aptamer. For the cancer cells and aptamers which bind specifically to altered surface molecules on cancer cells without the exact knowledge on changed sequences Cell-SELEX is used and in the field of abdominal surgery, researches with aptamers were performed on pancreatic, colorectal and liver cancer. The selected aptamers could target certain biomolecules or inhibit receptors that are involved in carcinogenesis. This characteristic is of particular value in pancreatic cancer, due to late diagnosis and insufficient treatment opportunities resulting in poor prognosis. Further researches are required, in order to determine, whether aptamers are capable of detecting biomarkers and inhibiting receptors or intrinsic pathways that are involved in carcinogenesis. So far, five promising approaches for cell detection seem to be: endogenous nucleic acid analysis [35].

APTAMER AND ANTIBODY IN CANCER

When compare to Monoclonal antibodies Aptamers, as “chemical antibodies”, are 15-20 times smaller. Though it is commonly used in clinic for cancer treatment due to its large in size effective penetration remains the matter of concern. The Insufficient penetration, inadequate drug distribution and lower intracellular concentration of drugs mainly cause Drug resistance and failure of treatment. In the year 2000, the comparative studies undertaken by Marolt et.al, between aptamers and antibodies (EpCAM aptamer with an EpCAM antibody in theranostic applications) of their tissue penetrating behavior. The final Comparative studies concluded on Penetration and retention result done in invitro three-dimensional tumorspheres and in vivo live animal imaging and mouse colorectal cancer xenograft model [36]. Resultant evidence found were that the EpCAM aptamer can not only effectively penetrate into the tumor sphere cores it can also be retained by tumor sphere cells for at least 24 h, while limited tumor penetration by EpCAM antibody was observed after 4 h incubation. As observed from in vivo live animal imaging, EpCAM aptamers displayed a maximum tumor uptake at around 10 min followed by a rapid clearance after 80 min, while the signal of peak uptake and disappearance of antibody appeared at 3 h and 6 h after

intravenous injection, respectively. The signal of PEGylated EpCAM aptamers in xenograft tumors was sustained for 26 h, which was 4.3-fold longer than that of the EpCAM antibody [37]. Also there were Consistent 1.67-fold and 6.6-fold higher accumulation of PEGylated aptamer in xenograft tumors than that of antibody, at 3 h and 24 h after intravenous administration, respectively. In addition, the aptamer achieved at least a 4-time better tumor penetration in xenograft tumors than that of the antibody at a 200 μm distances from the blood vessels 3 h after intravenous injection. All these data indicate that aptmers are superior to antibodies in cancer theranostics for their better tumor penetration, large homogeneous distribution and wide retention in tumor sites and concluded aptamers the better promising agents against targeted tumor therapeutics and molecular imaging [38].

HSP70 A CYTOPROTECTIVE FACTOR

HSP70 is a cytoprotective factor also known as Stress-inducible heat shock protein 70 (HSP70). It functions as an ATP-dependent chaperone, assisting the folding of newly synthesized proteins and polypeptides, the assembly of multi protein complexes, and the transport of proteins across cellular membranes .over expression of HSP70 inhibits apoptosis and lead to oncogenic transformation. Studies found that HSP70 over expression increases the tumorigenicity of cancer cells in rodent models and correlates with poor prognosis in cancer [39]. HSP70 has been demonstrated to bind to aptamers thereby preventing the recruitment of procaspase-9 to the apoptosome [40] Moreover, HSP70 can inhibit apoptosis by directly neutralizing the caspase-independent death effectors apoptosis inducing factor (AIF) [41] Targeting HSPs is an emerging concept in cancer therapy. Different inhibitors of HSP90 are being tested in clinical trials. These are mainly compounds derived from the geldanamycin antibiotic, such as the 17-allylamino-17 demethoxygeldanamycin (17AAG), but they also include synthetic small molecules designed to bind the ATP domain of HSP90 [42]. Like the synthetic molecules, geldanamycin derivatives also associate with the HSP90 ATP domain, thus inhibiting ATP binding and therefore affecting the function of signaling proteins whose structure depends on the HSP90 chaperone activity [43]. Currently 17AAG is being tested for its chemo sensitizing effects in phase III clinical trials with encouraging results in multiple myeloma [44]. Conversely, HSP70 down regulation is sufficient to kill tumor cells or to sensitize them to apoptosis induction in vitro and can reduce tumorigenicity in vivo. The anti apoptotic function of HSP70 involves interactions with several components of the apoptotic machinery. HSP70 can be targeted by a "negative" strategy, that is, siRNAs or antisense oligo nucleotides to down regulate its expression [45]. In addition, a "positive" HSP70-targeting, chemo sensitizing strategy in which a molecule that antagonizes HSP70 at the protein level is introduced into cancer cells [46].

APTAMERS SPECIFIC FOR CELL SURFACE BIOMARKERS

Cell surface biomarkers plays a vital role in biological processes, every signal has to pass through the surface of cell before reaching the destination many times dysfunction in regulations results due to signal impairment. Signal transduction, cell adhesion and migration, cell–cell interactions and communication between the intra- and extra-cellular environments are among the important role which depends completely on cell surface biomarkers. An abnormal expression of cell surface biomarkers is often related to tumorigenesis [47]. Clinically 60% of cancer therapy is done by targeting cell surface biomarkers by cancer-targeting drugs including therapeutic antibodies and small molecule inhibitors, target cell surface biomarker [48]. Till date many aptamers targeting cell surface biomarkers have been developed through the advancement of either the protein or cell-based SELEX technologies. These aptamers have been extensively studied for diagnosis and/or treatment of hematological malignancies [49] lung, [50] liver, [51] breast, [52] ovarian, [53] brain, [54] colorectal, [55] and pancreatic cancers, [56] as well as for identification and characterization of CSCs [57].

APTAMER-DRUG CONJUGATES

Aptamer-drug conjugation (ApDC) is a very simple yet effective model of non-covalently or covalently conjugating aptamer sequences directly with therapeutic agents. aptamer-conjugated Doxorubicin(Dox), is a chemotherapeutic agent extensively used in the treatment of various types of cancers, recently the cancer studies has reported that using aptamers along with Dox have enhanced therapeutic efficacy, when compared with tested Dox alone. Dox cytotoxicity is caused by its intercalation into the nucleic acid structure at the preferred paired CG or GC sites with subsequent inhibition of cancer cell proliferation. Dox can be non- covalently conjugated to oligo nucleotide aptamers containing CG/GC sequences through a simple incubation step. A recent report by Subramanian *et al.* describes the effectiveness of aptamer-Dox conjugates in the treatment of retinoblastoma [58]. In their study, a 2′-fluoro modified RNA aptamer EpDT3 (specific for EpCAM, a CSC marker) was non-covalently conjugated with Dox. After binding to EpCAM molecules expressed at the cancer cell surface, the EpDT3-Dox conjugates were preferentially internalized by the cancer and not by the healthy cells, greatly enhancing therapeutic efficacy and reducing treatment-associated side effects. Several other studies also utilized aptamer-Dox conjugates for cancer

therapy, such as HER2 aptamer-Dox conjugates targeting breast cancer [59] MUC1 aptamer-Dox conjugates targeting lung cancer [60] and PSMA aptamer-Dox conjugates targeting prostate cancer.

APTAMERS BASED BIOSENSORS

Conformational changes offers great flexibility and stability. In designing biosensor target binding is the key feature which is available in aptamer, for it undergoes significant conformational changes upon target binding. Monoclonal antibody being the recent advantageous trend in biotechnology field, still find hard to completely fill the needs of scientist due to various drawbacks one among them is use of animal and animal immune cells for production and preparation. Unlike monoclonal antibody aptamers scientist found that it can fit well for it do not require any culture and production compared to previous. This is also the reason behind aptamer based biosensor development instead of traditional antibodies.

Aptamers exhibit various advantages in biosensing, the small size, easy and convenient in production, makes them chemically stable and cost effective. Combining aptamers and nano material, effect was found significantly higher. Moreover it also offers remarkable flexibility and convenience in the design of their structures, which has led to novel biosensors that have exhibited high sensitivity and selectivity [61].

APTAMER-MEDIATED GENE THERAPY

Small interfering RNA (siRNA) and microRNA (miRNA) molecules are powerful gene silencing tools but due to limited in use in clinical perspective is due to lack of cell/tissue specificity during *in vivo* delivery. With various clinical trials and study it is been reported that combination of aptamers enhance target specificity and provide better result when compare to used alone. Similar to conjugation of chemotherapeutic agents, siRNA and miRNA can be covalently conjugated with aptamers to form aptamer-siRNA or aptamer-miRNA chimeras. McNamara *et al.* first developed an aptamer-siRNA chimera in which either Plk1 (polo-like kinase 1) siRNA or Bcl2 (B-cell lymphoma-2) siRNA were covalently conjugated with a modified A10 RNA aptamer against PSMA.100 Their results showed that this simple conjugation did not affect biological functions of either the aptamer or the siRNA [62].

PEGAPTANIB VEGF-SPECIFIC APTAMER

"Pegaptanib" VEGF-specific aptamer the only approved aptamer among many that undergoing clinical trials, approved by US food and drug administration on December 2004, except the smallest isoform VEGF121 it binds to all human VEGF isoform. Applications of aptamers is vast and used against distinct targets such as metal ions (k^+, Hg^{2+} and Pb^{2+}) organic dyes, peptides and proteins (thrombin, growth factor and HIV-associated peptides) various Small organic biomolecules AA, ATP, antibodies, vitamins, cocaine and even whole cells [63].

CONCLUSION

Aptamer is one among the precious compound identified and it is a worldwide promising class of compound; it is nearly very recent trend. still with enormous research and work only clinical trials has been concluded, it must come out for betterment of human population in market with easily availability, till now only one aptamer "pegaptanib" has been approved by US food and drug administration in December 2004 and is currently marketed by Pfizer and eyetech as macugen.Even with extensive research there are still widely spread disease and disorder we are unable to eradicate it completely. Many important promising compound chemical, physical as well as biological could not compete with one another each one is effective against its target. But then every compound identified obtain certain limitations, though aptamer is a recent trend it can be achieved high due to advantage of chemical modification, There is lot more to find about aptamer and their applications, various different works been undertaking against various disease and disorders using aptamers In brief to conclude apatmer then there is still a long way to go. The use of aptamers as therapeutic agents is still in its early stage of development. However, the Innovation and flexibility of SELEX methodology will allow aptamer technology to become a major player as an alternative approach in the battle against various diseases and disorder specifically viral diseases.

REFFERENCES

[1] Moore, M.D, Cookson, J. Coventry, V.K. Sproat, B. Rabe, L. Cranston, R.D. McGowan, I. James, (**2011**) *J. Biol. Chem.,* 286, 2526
[2] James, W. *J. Gen. Virol*. (**2007**), 88, 351–364.

[3] Wiegand, T.W., Williams, P.B., Dreskin S.C., Jouvinm, M.H., Kinet, J.P. and Tasset, D. (**1996**). *Journal of Immunology*. 157, l221–230
[4] Ellington AD and Szostak JW(**1990**). *Nature* ; 346: 818-822.
[5] Willis, M.C., Collins, B., Zhang, T., Green, L.S., Sebesta,D.P., Bell, C., Kellogg, E., Gill, S.C., Magallanez, A.,Knauer, S., Bendele, R.A., Gill, P.S., and Janjic, N. (**1998**). *Bioconjug. Chem*. 9, 573–582.
[6] Gong Q, Wang JP, Ahmad KM, Csordas AT, Zhou JH, et al. (**2012**) *Anal Chem* 84: 5365–5371.
[7] Balamurugan S, Obubuafo A, Soper SA, McCarley RL, Spivak DA. *Langmuir* (**2006**) ;22:6446–6453.[PubMed: 16800712]
[8] Baldrich E, Restrepo A, O'Sullivan CK. (**2004**) *Anal Chem*;76:7053–7063. [PubMed: 15571359]
[9] Bock LC, Griffin LC, Latham JA, Vermass EH, Toole JJ (**1992**) *Nature*;355:564–566. [PubMed:1741036]
[10] Subramanian Balamurugan, Anne Obubuafo, Robin L. McCarley, Steven A. So`per, and David A. Spivak(2008)Anal Chem; 80(24): 9630–9634. doi:10.1021/ac8009559. Effect of Linker Structure on Surface Density of Aptamer Monolayers and their Corresponding Protein Binding Efficiency
[11] Klussmann, Sven,(**2006**) The Aptamer Handbook. Wiley-VCH Verlag GmbH&Co., KGaA;Weinheim
[12] Adali, A.M., Paul, A, Wilhem., N. Ziemer, G. and Wendel, H.P. (**2001**) *Molecules*. 15, 1-11.
[13] Mazars, G.R. and Theillet, C. (**1996**). Direct sequencing by thermal asymmetric PCR. *Oligonucleotides* 117–128 (**2009**).
[14] Blake, C. M. *et al. Oligonucleotides* 117–128 (**2009**).
[15] Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, et al. (**2000**) *Nat Cell Biol*;2:469–75.
[16] Higuchi, R.G. and Ochman, H. (**1989**). *Nucleic Acids Research.* 17, 5865.
[17] Williams, K.P. and Bartel, D.P.(**1995**). *Nucleic Acids Research*. 23, 4220–4221.
[18] Guo P (**2010**) *Nat Nanotechnol*; 5: 833-842
[19] Ng EW and Adamis AP.(**2006**) Anti-VEGF aptamer (pegaptanib) therapy for ocular vascular diseases. Ann N Y Acad Sci; 1082: 151-171.
[20] Levy-Nissenbaum E, Radovic-Moreno AF, WangAZ, Langer R and Farokhzad OC(**2008**) *Trends Biotechnol*; 26: 442-449..
[21] Brody, E.N. and Gold, L. (**2000**) *Mol. Biotech.* 74, 5^13.
[22] Gold L, Polisky B, Uhlenbeck OC, Yarus M (**1995**) *Annu Rev Biochem* 64:763–797
[23] Joshi, P.J.; Fisher, T.S.; Prasad, V.R. (**2003**) *Curr. Drug Targets Infect. Disord.* 2003, 3, 383–400.
[24] McCabe, P.C.(**1999**). In PCR Protocol: A Guide to Methods and Applications, Edited by Innis, M. A., Gelfand, D. H.,Sninsky, J. J. & White, T. J. (Academic, New York), pp.76–83
[25] Kohn, D.B., et al. (**1999**). *Blood*. 94:368–371,
[26] Kolibaba, K.S. and Druker, B.J. (**1997**) *Biochim. Biophys. Acta* 1333, 217^248.
[27] Marolt U1, Cencic A, Gorenjak M and Potrc S (**2000**) Generating Aptamers for Cancer Diagnosis and]herapy 1Department of Abdominal and General Surgery, UKC Maribor, Ljubljanska 5, Maribor, Eu-slovenia
[28] Lee, S.W., and Sullenger, B.A. **1997**. *Nat. Biotechnol*. **15**:41–45.
[29] Chang SS, Reuter VE, Heston WD, Bander NH, Grauer LS, et al. (**1999**) *Cancer Res* 59: 3192- 3198.
[30] Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, et al. (**2007**) *Nano Lett* 7: 3065-3070.
[31] Cao Z, Tong R, Mishra A, Xu W, Wong GC, et al. (**2009**) *Angew Chem Int Ed Engl* 48: 6494-6498.
[32] Wu Y, Sefah K, Liu H, Wang R, Tan W (**2010**) *Proc Natl Acad Sci U S A* 107: 5-10
[33] Chu TC, Marks JW 3rd, Lavery LA, Faulkner S, Rosenblum MG, et al. (**2006**) Aptamer:toxin conjugates that specifically target prostate tumor cells. Cancer Res 66: 5989-5992 Department of Chemistry and Center for Biomodular Multi-Scale Systems, Louisiana State University, Baton Rouge, LA 70803
[34] Orava EW, Cicmil N, Gariépy J (**2010**) *Biochim Biophys Acta* 1798: 2190-2200.
[35] Ring A, Smith IE, Dowsett M (**2004**) *Lancet Oncol* 5: 79-88. flow cytometry analisis
[36] Dongxi Xiang et.al, (**2015**); Superior Performance of Aptamer in Tumor Penetration over Antibody: Implication of Aptamer-Based Theranostics in Solid Tumors
[37] Muchekehu R, Liu D, Horn M, et al.(**2013**) *Transl Oncol*; 6 (5):562-72.
[38] Minchinton AI, Tannock IF (**2006**). *Nat Rev Cancer*; 6 (8):583-92.
[39] Gilbert, J. C. *et al. Circulation* **116**, 2678–2686 (**2007**).
[40] Que-Gewirth NS and Sullenger BA(**2007**). *Gene Ther*; 14: 283-291
[41] Katherine Germer, Marissa Leonard, Xiaoting Zhang(**2012**) RNA aptamers and their therapeutic and diagnostic Applications Department of Cancer Biology, Vontz Center for Molecular Studies, University of Cincinnati College of Medicine, OH 45267
[42] LI, N., Wang, Y.,Pothukuchy, A., Syrett, A., Husain, N., Gopalakrisha, S., Kosaraju, P., And Ellington, A.D. (**2008**). *Nucleic Acids Res.* **36,** 6739–6751.

[43] Cho M, Xiao Y, Nie J, Stewart R, Csordas AT, et al. (**2010**) Quantitative selection of DNA aptamers through microfluidic selection and high-throughput sequencing. Proc Natl Acad Sci U S A 107: 15373–15378.
[44] Lee, S. W. & Sullenger, B. A. *Nature Biotech.* **15**, 41–45 (**1997**).
[45] Ruckman. J., Gold, L., Stephens. A. & Janjic, N. Nucleic acid ligands to integrins. US Patent 7,094,535 (**2006**).
[46] Anne-Laure R_erole, Jessica Gobbo, Aurelie De Thonel, Elise Schmitt, Jean Paul Pais de Barros1,Arlette Hammann, David Lanneau, Eric Fourmaux, Oleg Deminov, Olivier Micheau, Laurent Lagrost, Pierre Colas, Guido Kroemer, and Carmen Garrido (**2011**) Peptides and Aptamers Targeting HSP70: A Novel Approach for Anticancer Chemotherapy; DOI: 10.1158/0008-5472.CAN-10-1443
[47] Parekh, P, Kamble, S, Zhao, N, Zeng, Z, Portier, BP and Zu, Y (**2013**). *Biomaterials* 34:8909–8917.
[48] Esposito, CL, Passaro, D, Longobardo, I, Condorelli, G, Marotta, P, Affuso, A (**2011**). *PLoS ONE* 6: e24071.
[49] Wang, FB, Rong, Y, Fang, M, Yuan, JP, Peng, CW, Liu, SP (**2013**). *Biomaterials* 34: 3816–3827.
[50] Kim, MY and Jeong, S (**2011**). *Nucleic Acid Ther* 21: 173–178.
[51] Van Simaeys, D, López-Colón, D, Sefah, K, Sutphen, R, Jimenez, E and Tan, W (**2010**). *PLoS ONE* 5: e13770. 53.
[52] Cerchia, L, Esposito, CL, Jacobs, AH, Tavitian, B and de Franciscis, V (**2009**). *PLoS ONE* 4: e7971
[53] Sefah, K, Meng, L, Lopez-Colon, D, Jimenez, E, Liu, C and Tan, W (**2010**). *PLoS ONE* 5: e14269.
[54] Dua, P, Kang, HS, Hong, SM, Tsao, MS, Kim, S and Lee, DK (**2013**). *Cancer Res* 73: 1934–1945.
[55] Sefah, K, Bae, KM, Phillips, JA, Siemann, DW, Su, Z, McClellan, S *et al*. (**2013**). *Int J Cancer* 132:2578–2588.
[56] Subramanian, N, Raghunathan, V, Kanwar, JR, Kanwar, RK, Elchuri, SV, Khetan, V (**2012**). *Mol Vis* 18: 2783–2795.
[57] Liu, Z, Duan, JH, Song, YM, Ma, J, Wang, FD, Lu, X *et al*. (**2012**). *J Transl Med* 10: 148.
[58] Hicke, B.J., Stephens, A.W., Gould, T., Chang, Y.-F., Lynott, C.K., Heil, J., Borkowski, S., Hilger, C.-S., Cook, G., Warren, S., And Schmidt, P.G. (**2006**). *J. Nucl. Med*. **47,** 668–678.
[59] Hu, Y, Duan, J, Zhan, et al., 2012 Q, Wang, F, Lu, X and Yang, X-D (**2012**). *PloS One* 7: e31970.
[60] Held, D.M.; Kissel, J.D.; Patterson, J.T.; Nickens, D.G.; Burke, D.H. *Front. Biosci*(**2006**). , 11, 89–112
[61] Usman, N., and Blatt, L.M. **2000**. Characterization and development of nuclease-resistant synthetic ribozymes as a new class of therapeutics. *J. Clin. Invest.* In press.
[62] Peng, L. *et al*. *Microsc. Res. Tech.* **70**, 372–381 (**2007**).
[63] Jayasena, S.D. (**1999**) *Clin. Chem*, 45, 1628–1650.