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DNAZYMES AS NOVEL THERAPEUTICS

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ABSTRACT

DNAzymes are the catalytic DNA molecules which are single stranded with enzymatic activity. Many deoxyribozymes catalyses DNA phosphorylation, DNA adenylation, DNA deglycosylation, porphyrin metallation, thymine dimer photo reversion and DNA cleavage, act as catalysts in asymmetric synthesis. 10–23 DNAzymes are known for their excellent catalytic efficiency. Similar to other antisense molecules, DNAzymes are prone to nucleolytic degradation in body fluids. To prolong the half-life of oligonucleotides for in-vivo usage, to enhance biostability, to reduce toxicity and improve target affinity modified nucleotides are usually incorporated. Even with certain advantages and limitations for DNAzymes, they find wide applications in medicine and therapeutics like in cardiovascular, cancer, anti-bacterials, pain, brain disorders etc. In present study we have focused on the structure of first DNAzyme, its advantages, and various applications of the DNAzymes.

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KEYWORDS

DNAzymes, Cardiovascular, Tumerogenic, ribozymes.

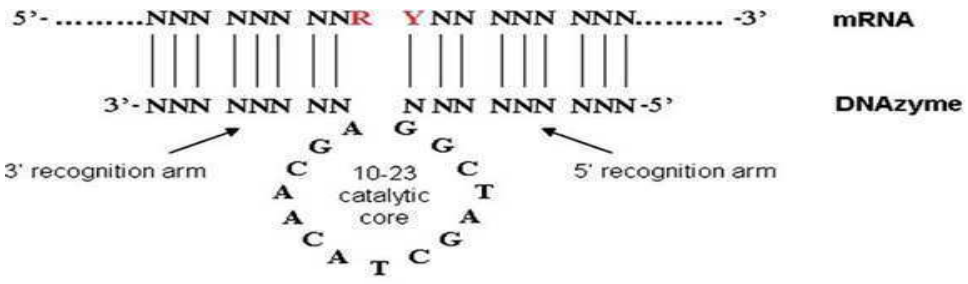
INTRODUCTION

Many gene knockdown techniques[1] have been explored, including antisense oligonucleotides, ribozymes, and decoys, short interfering RNAs (siRNAs) and DNAzymes. Disadvantages of phosphorothioate linkages and aptamers are avoided by all DNA-based phosphodiester-linked DNAzymes[2]. RNAzymes are more susceptible to degradation and antisense molecules interfere with nonspecific proteins due to the formation of G-quartets[3]. These types of disadvantages are not shown by catalytic DNAmolecules. DNAzymes are DNA molecules with catalytic action also called as DNA enzymes, catalytic DNA or deoxyribozymes. DNAzymes are single-stranded oligo deoxy nucleotides with enzymatic activity. No DNAzymes are known in nature[4].

HISTORY OF THE DNAZYMES

The first deoxyribozyme was discovered in 1994 by Professor Ronald R. Breaker[5]. Many deoxyribozymes catalyses are DNA phosphorylation, DNA adenylation, DNA deglycosylation, porphyrin metallation, thymine dimer photoreversion and DNA cleavage. Due to the chirality of DNAzyme one can use these DNAzymes as catalysts in asymmetric synthesis (the synthesis of chiral molecules from an achiral source). First isolated DNAzyme was '10–23' DNAzyme. It was initially described by Santoro and Joyce[6] in 1997 & 1998 and was so named as it was derived from the 23rd clone from the 10th round of PCR. The 10–23 DNAzyme is composed of a catalytic domain of 15 deoxyribonucleotides flanked by two substrate recognition arms. It will cleave any RNA substrate between an unpaired purine (A, G) and a paired pyrimidine (U, C) in the presence of Mg^{+2}. The 10–23 DNAzyme has excellent catalytic efficiency. The general structure of the 10–23 DNAzyme is shown in Figure 1. The 10–23 DNAzyme has many advantages over other antisense drugs[7-12]. Unlike antisense oligonucleotides, 10–23 DNAzyme not only binds the target RNAs but also cleaves them. Kurreck *et al*[13]., showed that 10–23 DNAzymes have a much higher cleavage activity than the ribozymes[13(a)]. In fact, its catalytic efficiency (k_{cat}/K_M) may reach $10^9 min^{-1}$, which is about 100-fold higher than that of the most active ribozyme. It also has a remarkable stability 1,00,000-fold as high as that observed with ribozyme under physiological conditions. Furthermore, 10–23 DNAzyme exhibits high flexibility for cleaving-site selectivity and substrate specificity, because it may cleave the purine–pyrimidine junctions of any RNAs and a single base mismatch in its antisense arms significantly decreases the cleavage activity. Professor Levon Khachigian[14] and his team at the Centre for Vascular Research at the University of New South Wales (UNSW) were the first to deliver DNAzymes into an animal model of human disease in 1999 and have defined the field of DNAzyme therapeutics.

Figure 1
The general structure of the 10–23 DNAzymes



The phosphodiester bond between the nucleotides shown in red is cleaved by 10–23 DNAzymes.

ADVANTAGES OF DNAZYMES

The following are few of the advantages[15] of DNAzymes.

- DNAzymes are stable in serum (3'-3'-linked inverted T) and are more stable than siRNA
- Synthesis is relatively inexpensive and easy
- Non-toxic in pre-clinical studies.
- Made entirely of phophodiester linkages, not phosphorothioate groups
- Self-sufficient catalysis and do not require the recruitment of catalytic cellular machinery (such as
- Dicer/RISC) to degrade target substrate.
- Increased stability both in-vitro and in-vivo
- DNAzymes are often more active
- Exhibits higher sequence specificity[15]

Similar to other antisense molecules, DNAzymes are prone to nucleolytic degradation in body fluids. To prolong the half-life of oligonucleotides for *in-vivo* usage, to enhance biostability, to reduce toxicity and improve target affinity modified nucleotides are usually incorporated[16]. LNA-DNAzymes[17] have LNA(locked nucleic acid] monomers introduced into the usual hybridizing arms, increasing the melting temperature of the molecule and enhancing binding affinity by conformational constraint placed on the sugar ribose ring. LNA-modified DNAzymes have higher catalytic rates and sensitivity to target than that of unmodified DNAzymes.

APPLICATIONS OF DNAZYMES:

(1) TREATMENT OF CARDIOVASCULAR DISEASES[18]

Despite improved pharmacotherapies and mechanical treatments, cardiovascular disease remains a principal cause of morbidity and mortality worldwide. Although gene therapies in the broad sense were proposed over two decades ago, there is now increasing interest in specific gene targeting using small molecule nucleic acid-based techniques. Numerous approaches are being suggested, based partly on novel technologies which are becoming rapidly available and partly by understanding the key genes that are responsible for cardiovascular function and dysfunction. ED5, Dz13, active TNF-α Dz, E2 and E4 are few DNAzymes that are used in cardiovascular diseases.

Table 1
DNAzymes and in vivo cardiovascular pathologies.

Target	DNAzyme	Model	Effect
Egr-1	ED3'-TAGCAGGTCCAGCA ACA TCCATCGGACCGGCGCC -5 (inverted 3' T)	1) Rat and pig ballon catheter injury 2) Rat myocardial ischemia reperfusion injury	Inhibition of neointima formation Attenuation of infarct size and inflammatory mediators
c-jun	Dz13'-TGTTGCGGAAG CA ACATCGATCGGAAGG A GGGC-5' (inverted 3' T)	Rat carotid artery ligation	Inhibition of neointima formation
TNF-α	Active TNFα Dz 3'-TsTsTsCsCsTsGsTsGs GAGCAACATCGGAsCs TsCsGsTsG-5'	Rat AMI by LAD ligation	Increased cardiac output
VDUP1	E4 3'-TCGAGTTAGAGCAAC A TCGATCGGACCACTAC-5' (inverted 3' T)	Rat AMI by LAD ligation	Decreased apoptosis and collagen expression, increased function

i) Egr-1 DNAzymes:

Early growth response (Egr-1) is a zinc finger transcription factor which is required for SMC (smooth muscle cell) and endothelial cell recovery from mechanical injury in vitro[19, 20]. SMC proliferation is a characteristic of neointima formation following angioplasty, A DNAzyme targeting the $A^{816}U$ junction within the start codon of rat Egr-1, denoted as ED5 inhibited the upregulation of Egr-1 protein and inhibited neointima formation. Early growth response factor-1 has also been recognized to play a role in the response to ischaemia–reperfusion injury in a variety of other cell types and organ systems. Outside the cardiovascular system, Egr-1 has been implicated in ischaemia–reperfusion responses in the lung, intestine and kidney. Egr-1 targeting DNAzymes are also known to inhibit angiogenesis by downstream repression of fibroblast growth factor-2.

ii) c-Jun DNAzymes :

The proto-oncogene c-Jun, a component of the activator-protein-1 (AP-1) transcription factor, has been the subject of much recent interest in the DNAzyme field. The role of c-Jun in a wide variety of disease processes has been unravelled using Dz13. Dz13 inhibited human, porcine and rat SMC proliferation *in vitro* and suppressed neointima formation in rats subjected to carotid artery ligation. Dz13 also suppressed proliferation and migration of human microvascular endothelial cells *in vitro*[21], which suggested an important regulatory role for c-Jun in angiogenesis. The protein content and proteolytic activity of matrix metalloproteinase-2, an enzyme that commonly degrades extra cellular matrix

proteins and basement membrane, was also repressed by Dz13 thus implicating matrix metalloproteinase-2 as a downstream transcriptional target of c-Jun. Extensive studies in murine models of inflammation showed that Dz13 inhibited vascular permeability, endothelial-monocytic adhesion, leukocyte adhesion and extravasation, and neutrophil infiltration[22].

iii) VDUP 1 DNAzyme:

DNAzymes have also been used to study proteins associated with increased oxidative stress due to myocardial ischaemia, in particular the vitamin D3-upregulated protein-1 (VDUP1). VDUP1 inhibits interaction between thioredoxin and apoptosis signal-regulated kinase-1 (ASK1). Targeting of VDUP1 by DNAzyme-E4 resulted in decreased VDUP1 mRNA levels and ASK1 activity with lower apoptotic levels[23].

iv) Other DNAzymes:

Tumour-necrosis factor-α is associated with atherosclerosis, AMI (acute myocardial infarction) and heart failure[24]. PAI-1(plasminogen activator inhibitor-1) is one of the marker of cardiovascular disease, levels of which are elevated in patients with restenosis, atherosclerosis and AMI. PAI-1 DNAzyme inhibited transforming growth factor β-mediated PAI-1 stimulation in endothelial cells and also decreased the PAI-1 expression. PAI-1 DNAzyme improves neovascularization, and is also decreased apoptosis in the peri-infarct region and improved cardiac function, as measured by myocardial ejection fraction.

2) CANCER TREATMENT BY VEGFR2 DNAZYMES

The vascular endothelial growth factor receptor (VEGFR) is an important angiogenic target for cancer gene therapy. Designing of an mRNA-cleaving oligodeoxynucleotide i.e (VEGFR2 DNAzyme) which targets the VEGF receptor 2 (VEGFR2) transcripts proved that this DNAzyme was digesting efficiently mRNA substrates of VEGFR2 in a concentration- and time-dependent manner and DNAzyme was also induces apoptosis and markedly inhibits endothelial cell growth. The DNAzyme in complex with a nonviral carrier also significantly inhibited tumor growth *in vivo*. Marked cell death in the peripheral regions of the tumor accompanied by a reduction in blood vessel density is consistent with the antiangiogenic[25] mechanism of the DNAzyme.

i) Treatment of APL by reducing the proliferation and by inducing apoptosis in APL cells:

Acute promyelocytic leukaemia (APL) is characterized by the t(15:17) translocation, which fuses the retinoic acid receptor a (RARa) gene on chromosome 17 to the promyelocytic leukaemia (PML) gene on chromosome 15 to produce the PML/RARa fusion gene. PML/RARa is critical in the development of APL. Targeting the PML/RARa fusion gene with DNAzymes (DZ1 and DZ3) can induce apoptosis in APL cells and may have a role in the treatment of APL[26]. Now a days DNAzymes are being employed in a variety of areas of research including restoration of drug sensitivity[27], inhibition of viral diseases such as HIV[28], and Hepatitis B[29], inhibition of neointima formation[30] after arterial injury, and direction against transformed phenotype products

ii) Study of the expression and/or activity of uPA or uPAR in tumor cells:

Proteolysis of extracellular matrix (ECM) and basement membrane is an essential

mechanism used by cancer cells for their invasion and metastasis. The ECM proteinases are divided into three groups: metalloproteinases, cysteine proteinases and serine proteinases. The urokinase plasminogen activator (uPA) system is one of the serine proteinase systems involved in ECM degradation. Members of this system, including uPA and its receptor (uPAR), are overexpressed in several malignant tumors. This system plays a major role in adhesion, migration, invasion and metastasis of cancer cells, thus making it an important target for anticancer drug therapy. Several strategies, including the use of antisense oligodeoxynucleotides, ribozymes, RNAi, uPA inhibitors, soluble uPAR, catalytically inactive uPA fragments, synthetic peptides and synthetic hybrids are under study, as they interfere with the expression and/or activity of uPA or uPAR in tumor cells. Use of DNAzymes is under investigation to combat the uPA activity in cancer.

3) ANTIBACTERIAL ACTIVITY

10–23 DNAzyme has the potential to suppress gene expressions through sequence-specific mRNA cleavage. 10–23 DNAzyme cannot replicate endogenously. Its dependence on exogenous delivery limits its applications. By cloning the 10–23 DNAzyme into the M13mp18 vector, two circular DNAzymes, C-Dz_7 and C-Dz_{482} are constructed and were targeted the β-lactamase mRNA. These circular DNAzymes show *in vitro* catalytic efficiencies (k_{cat}/K_M) of 7.82×10^6 & 1.36×10^7 M^{-1}. min^{-1}, respectively. Their dependence on divalent metal ions is similar to that found with linear 10–23 DNAzyme. Importantly, the circular DNAzymes are not only capable of replicating in bacteria but also exhibit high activities in inhibiting β-lactamase[31] and bacterial growth.

Table 2
Kinetic parameters of the circular and linear DNAzymes'

DNAzyme	k_{cat} (min^{-1})	K_M (nM)	k_{cat}/K_M ($M^{-1}\cdot min^{-1}$)
C-Dz_7	0.86 ± 0.15	110.1 ± 25.3	7.82×10^6
C-Dz_{482}	1.07 ± 0.21	78.6 ± 11.8	1.36×10^7
L-Dz_7	1.24 ± 0.17	42.3 ± 1.9	2.93×10^7
L-Dz_{482}	1.21 ± 0.08	31.7 ± 4.1	3.82×10^7
Denatured C-Dz_{482}	2.27 ± 0.61	19.5 ± 9.3	1.16×10^8

Kinetic parameters of the DNAzymes were obtained through analysis of the DNAzymes under multiple turnover conditions with an excess amount of the substrates in 50 mM of Tris–HCl (pH 7.6) and 10 mM of $MgCl_2$. The initial velocities at each substrate concentration are used to generate an Eadie–Hofstee plot. The k_{cat} and K_M values are deduced from

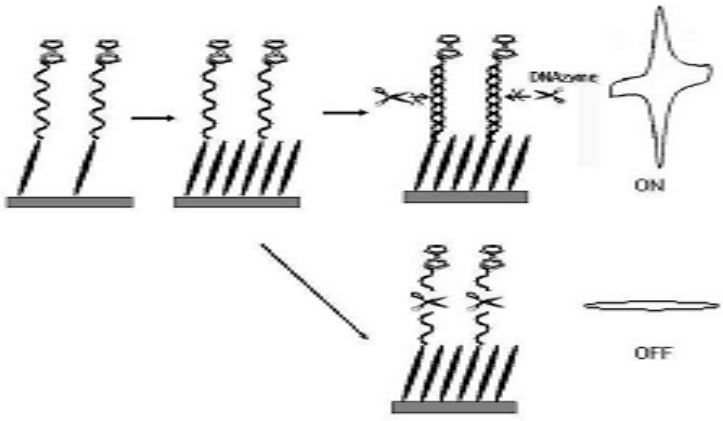
the *y* intercepts and slopes, respectively. All the data represent mean ± SD of three replicates.

4) ELECTROCHEMICAL DNA DETECTION

Electrochemical techniques have experienced substantial growth as promising tools for detection of biomolecules in molecular diagnosis, environmental monitoring, or food authentication. The detection of DNA hybridization among them is of significantly scientific and technological importance in chip-based characterization of gene expression patterns and detection of pathogens. Use of DNAzymes is a new method using enzymatic cleavage of immobilized DNA. The strategy is based on that DNAzyme (nuclease S1) is able to specifically cleave only single strand DNA (ssDNA), not double strand DNA

Figure 2

Schematic illustration of the electrochemical DNA detection using DNAzyme. (dsDNA).



Electro active ferrocene group linked to thiolated ssDNA and synthesized capture is immobilized on gold electrode. Then, target DNA is hybridized and nuclease S1 cleaves non hybridized DNA probe. The difference of nuclease S1 enzymatic cleavage on single and double strand modified gold electrode is characterized by Cyclic Voltametry (CV). Characterization of the modified surface by nuclease S1 is done through the mass change with surface plasmon resonance (SPR) experiments. If one uses DNAzymes there is no need for the post hybridization treatment with either hybridization indicators or other exogenous signaling molecules which most of the electrochemical hybridization detection systems require[32].

5) IDENTIFICATION OF ACCESSIBLE SITES IN ANY TARGET RNA FOR ANTISENSE OLIGONUCLEOTIDES-BASED GENE INACTIVATION METHODS

Identification of accessible sites in targeted RNAs is a major limitation to the effectiveness of antisense oligonucleotides. The “10-23” DNA enzyme or DNAzyme, which is a small catalytic DNA, has been shown to efficiently cleave target RNA at purine-pyrimidine junctions[33] in vitro in hepatitis C virus nonstructural gene 3 (HCV NS3) RNA that encodes viral helicase and protease. Optimized DNAzyme DH5 E can now be used in cell culture and *in vivo* to inhibit virus replication.

6) IN PAIN THERAPY

Vanilloid receptor subtype 1 (VR1) which is also designated as Transient Receptor Potential Channel V1, is a cation channel, which can be activated by capsaicin, heat and protons. It plays an important role in pain perception and is thought to be a new target for pain therapy. A DNAzyme called DV15 E4 was developed to investigate the role of the vanilloid receptor for nociception; soon we may use DNAzymes in pain therapy[34] also.

7) DNAZYMES IN BRAIN DISORDERS

The use of ribozyme and DNAzyme strategies were also introduced in various brain disorders. Huntington's, Alzheimer's and Parkinson's are some of brain diseases. Huntington's disease (HD) is a progressive brain disorder that causes uncontrolled movements, emotional problems, and loss of thinking ability. The underlying cause of Huntington's disease is the inheritance of a copy of the gene encoding huntingtin with an expanded polyglutamine-encoding CAG repeat located within the 5′ end of the coding region. The mutant huntingtin protein is expressed during development through adulthood, causes neuronal dysfunction, and ultimately cell death of neurons in the striatum. The neuropathology is present to a varying extent in other regions of the brain. Yen et al. demonstrated the first effective destruction of the mutant huntingtin mRNA using a specific DNAzyme that was able to cleave the mutant huntingtin mRNA in a sequence-specific manner[35], that lead to significant reduction of mutant huntingtin protein expression in mammalian cells. Therefore, with the reduction of the mutant huntingtin, several pathways associated with huntingtin are altered and less cellular toxicity is observed. So, one can use DNAzymes to significantly reduce huntingtin levels and to reduce the severity of the disease in near future.

8) DNAZYMES AS DIAGNOSTIC TOOL

In the diagnostic pathology laboratory[36], immunohistochemistry (IHC) is used to localize tissue proteins. After an antibody binds a protein of interest, additional antibodies and biotin avidin or polymer reagents are used to bring a chromogenic enzyme to the site of the protein of interest. Most commonly, this chromogenic enzyme is horseradish peroxidase (HRP), used because it catalyzes the oxidative polymerization of 3,3′-diaminobenzidine (DAB) into an insoluble brown precipitate. In an effort to develop alternative reagents for tissue protein detection assays, oligonucleotide aptamers are proposed. Aptamers are short single-stranded oligonucleotides, DNA or RNA, that are selected through an iterative process (SELEX) from random sequence RNA/DNA pools, and that can bind to other molecules with high affinities and selectivity. DNA aptamers have numerous practical advantages over protein reagents like antibodies and enzymes, such as highly parallel and automated discovery and production, relatively simple derivatization chemistries, and stability to dry and storage under ambient conditions.

9) DNAZYMES IN MUSCLE DISORDERS

Ribozymes and DNAzymes have been widely used towards the better understanding and therapy of various skeletal and smooth muscle disorders[37]. Myotonic dystrophy type I, SCCMS, myotonia congenita and neuromuscular

junction abnormalities, restenosis of coronary arteries and hypertension are some of the disorders that scientists introduced the use of ribozymes and DNAzymes. SCCMS (slow channel congenital myasthenic syndrome) is a dominant genetic disorder which is progressive and incurable. It is thought that prolonged activation of the Ach receptors, due to the mutations, leads to excess calcium entry resulting in an "endplate myopathy" and muscle weakness. Thus SCCMS is an example of a dominant excitotoxic disorder caused by "gain of function" mutations. At the neuromuscular junction, the safety margin for neuromuscular transmission is only compromised if AChR levels fall below around 30% of normal. The muscle acetylcholine receptor (AChR) is expressed at the neuromuscular junction, and plays the principal role in nerve to muscle signal transmission. Abdelgany et al. designed hammerhead ribozymes in order to target RNA transcripts from four different slow channel congenital myasthenic syndrome mutations. These hammerhead ribozymes are able to efficiently discriminate between mutant and wild type RNA transcripts that differ only by a single nucleotide substitution. Furthermore, the ability of DNAzymes to cause allele-specific cleavage in transcripts where the mutation creates a putative cleavage site or full DNAzyme:target binding was tested. Alelle-specific cleavage is demonstrated in both cases under simulated physiological conditions.

10) DNAZYMES IN HYPERTENSION

Angiotensin II (Ang II) plays an important role in the development of hypertension and atherosclerosis by inducing vascular smooth muscle cell growth and synthesis of aldosterone. Activation of Leukocyte-type 12-lipoxygenase (12-LO) has been proposed to be an important mechanism for AngII by inducing hypertrophy of vascular smooth muscle cells. This finding prompted scientists to design a chimeric RNA hammerhead ribozyme[38] against the first GUC sequence at nucleotide 7 of porcine leukocyte 12-LO mRNA. The ribozyme was transfected into porcine aortic vascular smooth muscle cells, causing a significant decrease of endogenous porcine leukocyte-type 12-LO mRNA and protein levels. Down regulation of 12-LO levels, have the potential to protect vascular smooth muscle cells from hypertrophy. The results from this study indicated the feasibility of using new ribozyme technology to study the specific effects of a gene pathway in vascular disease and the potential *therapies.*

11) DNAZYMES IN WASTE WATER TREATMENT

Microbes play an important role in bioremediation, wastewater treatment, waste gas treatment, and solid waste treatment[39, 40]. Filamentous bacterium *Sphaerotilus natans* is one of the microbe used in waste water treatment. The quantification of 16S rRNA derived from *Sphaerotilus natans* is of considerable interest because the amount of 16S rRNA is directly proportional to the growth rate of the cell[41, 42] and has been considered an indicator of bacterial activity[43]. A variety of methods have been used to identify and quantify the microbes playing a key role in environmental processes and treatment systems[44]. Till date the

quantification of 16S rRNA is usually done by quantitative PCR (competitive and real-time), reverse transcription or dot blot hybridization. Real-time PCR requires expensive instruments and reagents. Competitive PCR and dot blot hybridization are laborious and time-consuming. Therefore a rapid and cost-effective method is required. By using a complex microbial community waste water is treated by process called activated sludge systems. One of the major operational problems of activated sludge systems is the excessive growth of filamentous bacteria, which causes poor sludge settling[45] (bulking). To improve the efficiency of the activated sludge systems, one has to avoid bulking caused by filamentous bacteria i.e. quantification of the 16S rRNA is needed for the early detection of the bulking. DzECO-24, DzPPU-24 and DzSNA-24 are DNAzymes developed for the quantification of the16S rRNA. Advantages of the DNAzyme method, no need of a standard curve or internal standard, at a time the content of the target rRNA is calculated from the amount of cleaved RNA and intact RNA i.e. less time and money. Time of the DNAzyme method is less than 4hours which comprises the extraction of RNA using a standard commercial kit (2 h), incubation for the DNAzyme reaction (1 h), and separation of the reaction products by electrophoresis and their detection (1 h). Therefore, the DNAzyme method[46] is more cost-effective and easier to perform as DNAzymes are more stable, easy to handle and the solution of DNAzyme can be preserved at room temperature. This method will be useful for measuring the activity of a specified bacterial species in the actively growing community used for an environmental biotechnology such as wastewater treatment. We may also use DNAzyme method in clinical microbiology to detect the activity of pathogenic bacteria.

12) DNAZYMES-NANOTECHNOLOGY

DNA molecules have been utilized both as powerful synthetic building blocks to create nanoscale architectures and as versatile programmable templates for assembly of nanomaterials. The functions of DNA molecules have been elaborated from pure genetic information storage to catalytic functions like those of protein enzymes (DNAzymes) and specific binding functions like antibodies (aptamers). DNA-nanotechnology[47] is a new interdisciplinary field which combines the functional DNA biology with nanotechnology to generate more dynamic and controllable DNA-based nanostructures or DNA-templated nanomaterials that are responsive to chemical stimuli. DNA nanotechnology derived devices can be used in many other fields for practical applications, such as sensing, environmental monitoring, medical diagnostics, drug screening, therapeutics, nanoelectronics, nanophotonics, and quantum computing.

CONCLUSION

By combining the DNAzymes and the aptamers (nucleic-acid-based binding molecules which are obtained by a combinatorial selection method known as systematic evolution of ligands by exponential enrichment (SELEX), in which DNA molecules with the desired binding properties are isolated from a library containing as many as 10^{15} random sequences) we will get **a** new class of functional nucleic acids known as allosteric DNAzymes or aptazymes. By using

them in near future, we can develop new therapeutic approaches to the dreadful diseases and DNAzymes will defiantly emerge as novel therapeutic agents.

REFERENCES

1. Bhindi R, Fahmy R G, Lowe HC, Chesterman C N, Dass C R, Cairns M J et al. Brothers in arms: DNA enzymes, short interfering RNA, and the emerging wave of small-molecule nucleic acid-based gene-silencing strategies. Am J Pathol, 171:1079–1088, (2007).
2. Sen D. and Geyer, C . R. DNA enzymes. Curr. Opin. Chem. Biol, 2: 680–687, (1998).
3. Stein CA. Controversies in the cellular pharmacology of oligodeoxynucleotides. Ciba Found Symp, 209 :79–89 ,(1997).
4. Silverman S. K. DNAzymes- DNA catalyst for bioorganic chemistry. Org. Biomol. Chem., 2: 2701, (2004).
5. Breaker R. R. Making catalytic DNAs. Science, 290: 2095–2096, (2000).
6. Santoro S.W. and Joyce, G. F. A general purpose RNA cleaving DNAzyme. Proc. Natl Acad. Sci. USA, 94:4262–4266, (1997).
7. Sun L.-Q., Cairns, M. J., Saravolac, E.G., Baker, A. and Gerlach, W.L. Catalytic nucleic acids: from lab to applications. Pharmacol. Rev, 52:325–347, (2000).
8. Santoro S.W. and Joyce, G.F. Mechanism and utility of an RNA cleaving DNA enzyme. Biochemistry, 37: 13330–13342, (1998).
9. Finkel E. DNA cuts its teeth as an enzyme. Science, 286: 2441–2442 ,(1999).
10. Dass C.R., Saravolac, E.G., Li, Y. and Sun, L.Q. Cellular uptake, distribution and stability of 10–23 Deoxyribozymes. Antisense Nucleic Acid Drug Dev, 12: 289–299, (2002).
11. Emilsson G.M. and Breaker, R.R. (2002) Deoxyribozymes: New activities and new applications. Cell. Mol. Life Sci, 59: 596–607, (2002).
12. Kong X.-D., Zhu, S.-Z., Gou, X.-J., Wang, X.-P., Zhang, H.-Y. and Zhang, J. A circular RNA–DNA enzyme obtained by in vitro selection. Biochem. Biophys. Res. Commun, 292: 1111–1115, (2002).
13. Kurreck, J., Bieber, B., Jahnel, R. and Erdmann, V. A. Comparative study of DNA enzymes and r ibozymes against the same full-length messenger RNA of the vanilloid receptor subtype I. J. Biol. Chem, 277: 7099-7107, (2002).
14. Khachigian L.M. DNAzymes: cutting a path to a new class of therapeutics. Curr. Opin. Mol. Ther, 4: 119–121, (2002).
15. Steffen Schubert, Volker A. Eadmann and Jens Kurreck, RNA cleaving '10-23' DNAzymes with enhanced stability and activity, Nucleic Acids Research, 31:5982-5992,(2003).
16. Kurreck, J. Antisense technologies—improvement through novel chemical modifications. Eur. J. Biochem , 270:1628–1644,(2003).
17. Vester,B., Lundberg,L.B., Sorensen,M.D., Babu,B.R., Douthwaite,S. and Wengel,J. LNAzymes: incorporation of LNA-type monomers into DNAzymes markedly increases RNA cleavage. J. Am. Chem. Soc, 124:13682–13683,(2002).
18. V. L. Benson, L. M Kachigian, H, C. Lowe, DNAzymes and cardiovascular disease, British Journal of Pharmacology,154:741-748,(2008).
19. Santiago FS, Atkins DG, Khachigian LM.

Vascular smooth muscle cell proliferation and regrowth after mechanical injury in vitro are Egr-1/NGFI-A-dependent. Am J Pathol, 155: 897–905, (1999a).

20. Santiago FS, Lowe HC, Day FL, Chesterman CN, Khachigian LM. Early growth response factor-1 induction by injury is triggered by release and paracrine activation by fibroblast growth factor-2. Am J Pathol, 154: 937–944,(1999b).
21. Zhang G, Dass CR, Sumithran E, Di Girolamo N, Sun LQ, Khachigian LM. Effect of deoxy ribozym -es targeting c-Jun on solid tumor growth and angiogenesis in rodents. J Natl Cancer Inst ,96: 683- 696,(2004).
22. Fahmy RG, Waldman A , Zhang G, Mitchell A, Tedla N, Cai H et al. Suppression of vascular permeability and inflammation by targeting of the transcription factor c-Jun. Nat Biotechnol, 24:856- 863, (2006).
23. Xiang G, Seki T, Schuster MD, Witkowski P, Boyle AJ, See F et al. Catalytic degradation of vitamin D up-regulated protein-1 mRNA enhances cardiomyocyte survival and prevents left ventricular remodeling after myocardial ischemia. J Biol Chem , 280: 39394–39402, (2005b).
24. Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB et al. Tumor necrosis factor- alpha and tumor necrosis factor receptors in the failing human heart. Circulation 93:704-711, (1996).
25. Leizang, Warren J.Gasper, Sanfold A. Strass, Olga Bloffe, Myrtle A .Davis and Ajames Mixson, Angiogenic inhibition mediated by a DNAzyme that targets vascular endothelial growth factor recep -tor-2, Cancer Research, 62: 5463-5469,(2002).
26. Majid Kabuli, John Ahman,,Liu Yin and Khamid Tobal, Targeting PML/RARα transcript with DNA -zyme results in reduction of proliferation and induction of apoptosis in APL cells, TheHematology Journal, 5: 426-433, (2004).
27. Matsushita H, Kizaki M, Kobayashi H, Ueno H, MutoA, Takayama N et al. Restoration of retinoid ensitivity by MDR1 ribozymes in retinoic acid-resistant myeloid leukaemia cells. Blood, 91: 2452- 2458, (1998).
28. Unwalla H, Banerjea AC. Inhibition of HIV-1 gene expression by novel macrophage-tropic DNA enzymes targeted to cleaveHIV-1TAT/REVRNA. Biochemical J , 357:147–155, (2001).
29. Beck J, Nassal M. Efficient hammerhead ribozyme mediated cleavage of the structured hepatitis B virus encapsidation signal in vitro and in cell extracts, but not in intact cells. Nucleic Acid Res, 23: 4954–4962, (1995).
30. Khachigian LM, Fahmy RG, Zhang G, Bobryshev YV, Kaniaros A. c-Jun regulates vascular smooth muscle cell growth and neointima formation after arterial injury. Inhibition by a novel DNA enzyme targeting c-jun. J Biol Chem , 277: 22985–22991, (2002).
31. Sandanayaka V.P. and Prashad, A.S. Resistance to beta-lactam antibiotics: structure and mechanism based design of beta-lactamase inhibitors. Curr. Med. Chem, 9: 1145–1165, (2002).
32. I. Ihara, Y. Maruo, S. Takenaka and M. Makoto, Nucleic Acids Res, 24: 4273, (1996).
33. Li Y et al. A Complex RNA - Cleaving DNAzyme That Can Efficiently Cleave a Pyrimidine- Pyrimidine Junction, J. Mol. Biol, 400:689–701, (2010).

34. Caterina, M.J., Schumacher, M.A., Tominaga, T.A., Rosen, T.A., Levine, J.D. and Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature, 389: 816–824,(1997).
35. Yen,L., Strittmatter, S.M. and Kalb, R.G. Sequence-specific cleavage of Huntingtin mRNA by catalytic DNA. Ann. Neurol, 46: 366–373,(1999).
36. Thirstrup D and Baird G S. Histochemical application of a peroxidase DNAzyme with a covalently attached Hemin Cofactor. Anal. Chem, 82: 2498–2504, (2010).
37. Mastroyiannopoulos N P et al. The Application of Ribozymes and DNAzymes in Muscle and Brain. Molecules, 15:5460-5472, (2010).
38. Thomson, J.B., Tusch l,T. and Eckstein,F. The hammerhead ribozyme. In Eckstein, F. and Lilley, D.M.J.(eds), Catalytic RNA. Springer-Verlag, Berlin, Germany,173–196,(1996).
39. Abraham, W.-R., B. Nogales, P. N. Golyshin, D. H. Piper, and K. N. Timmis. Polychlorinated biphenyl -degrading microbial communities in soils and sediments. Curr. Opin. Microbiol, 5: 246-253, (2002).
40. Furukawa, K., H. Suenaga, and M. Goto.Biphenyl dioxygenases: functional versatilities and directed evolution. J. Bacteriol, 186: 5189–5196, (2004).
41. Nogales, B., E. R. B. Moore, E. Llobet-Brossa, R. Rossello-More, R., Amann, and K. N. Timmis. Combined use of 16S ribosomal DNA and 16S rRNA to study the bacterial community of Polychlorinated biphenyl-polluted soil. Appl. Environ. Microbiol, 67: 1874–1884 , (2001).
42. Poulsen, L., G. Ballard, and D. A. Stahl. Use of rRNA fluorescence in situ hybridization for measuring the activity of single cells in young and established biofilms. Appl. Environ. Microbiol. 29: 1354–1360 ,(1993).
43. McKillip, J. L., L. A. Jaykus, and M. Drake. rRNA stability in heat killed and UV-irradiated enterotoxigenic Staphylococcus *aureus* and Escherichia coli O157:H7. Appl. Environ. Microbiol. 64: 4264-4268, (1998).
44. Wilson, M. S., C. Bakerman, and E. L. Madsen. In situ, real-time catabolic gene expression: extraction and characterization of naphthalene dioxygenase mRNA transcripts from groundwater. Appl. Environ. Microbiol. 65: 80–87, (1999).
45. Wagner, M., R. Amann, P. Kampfer, B. Assmus, A. Hartmann, P. Hutzler, N. Springer, and K. H. Schleifer. Identification and in situ detection of gram-negative filamentous bacteria in activated sludge. Syst. Appl. Microbiol., 17: 405–417 , (1994).
46. Hikaru Suenaga, Rui Liu, Yuko Shiramasa and Takahiro Kanagawa, Novel approach to quantitative detection of specific rRNA in a microbial community using catalytic DNA, Applied and Environmental Microbiology, 71(2005),4879-4884.
47. Lu Y. and Liu J. Functional DNA nanotechnology: emerging applications of DNAzymes and aptamers. Current opinion in Biotechnology,17: 580-588,(2006).