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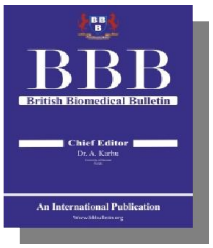

Original

Antisense Technology: Oligonucleotides and its Delivery Strategies

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ARTICLE INFO

Received 20 Jan. 2014
Received in revised form 21 Jan. 2014
Accepted 22 Jan. 2014

Keywords:
Antisense,
Oligonucleotide,
Oligonucleotide Chemistry,
Cell Penetrating Peptides,
Nano particular Oligonucleotide delivery.

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ABSTRACT

Aim: The present study highlights antisense technology where oligonucleotides have predominant role in its delivery for various diseases. **Objective:** Antisense compounds are biological molecules consisting of small ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) segments (Oligonucleotide) which have enormous potential for the treatment of number of diseases. **Methods:** All the methods analyzed were obtained through high impact articles. **Results:** Several impediments were made to Oligonucleotide for its widespread usage as them as drugs to overcome their lack of stability in physiological fluids and their poor penetration into cells. Experiments like, association with or encapsulation within nano-and micro sized drug delivery systems, lipids and polymers could help to increase the efficiency of oligonucleotide delivery. This review discusses the use of cationic lipids, Cell penetrating peptides, nanoparticles, microparticles and several novel methods that have recently been explored as delivery vehicles. **Conclusion:** The use of naked Oligonucleotide given a clear cut way to new targets for potential therapeutic compounds. Apart this, antisense therapy represents a promising and evolving approach to the management of numerous diseases in which a specific abnormality has been identified as a primary or major contributing etiologic factor. In near future, authors strongly believe that antisense therapy will give a positive way for number of *In vitro* experiments and break through for many life threatening diseases.

Introduction

Basic medical and genetic research in recent years has shown many incurable diseases are caused by defective regulation of certain genes[1].This manifests as excessive or faulty synthesis of proteins that can trigger diseases and promote their progression. The use of antisense molecules to inhibit the synthesis of such pathogenic proteins represents an innovative therapeutic approach[2]. Normally gene expression is the results of molecular changes of the protein. For this the cell must undergo two major complex processes like transcription and translations. During this process a copy of RNA (Ribo nucleic acid) is made from DNA (Deoxyribo nucleic acid) with the involvement of many enzymes like as polymerase, helicase, exonuclease, ligase. These enzymes unwind the double helix base pairs of DNA and direct this to match with RNA base pairs with help of single stranded binding protein, results heterogeneous nuclear RNA (hnRNA). This particular hnRNA spliced, Protected from cellular environment and shifted from nuclear membrane to cytoplasm, immediately triggers the protein production by hooking up with ribosomes[3].Nucleic acid is termed an "anti-sense" because of its base sequence is complementary to the gene's messenger RNA (mRNA), which is called the "sense" sequence (so that a sense segment of mRNA " 5'-AAGGUC-3' " would be blocked by the anti-sense mRNA segment " 3'-UUCCAG-5' "). The use of antisense oligo deoxynucleotides (ODNs) both in research and therapy have emerged as powerful alternative or complement to small molecule inhibitors employing traditional drug design strategies. As ODNs are short pieces of synthetic and chemically modified DNA designed to hybridize to specific mRNA sequences. They inhibit gene expression mainly through RNase-H activation or hybrid arrest, steric blockage of translation[4,5]. They are being explored as potential therapeutic tools against viral infections, cardiovascular, inflammatory and hematological diseases and cancer[6]. Occasionally, a bad mRNA molecule is synthesized so that the resulting protein cannot function properly. Abnormalities of proteins cause many diseases that afflict humans. Therefore, it seems logical to conclude that if the expression of these malfunctional proteins (Figure 1) could be stopped, the sources of disease would be obliterated and the disease will be treated, if not cured.

The first report of usage of this particular antisense oligodeoxy nucleotides to inhibit *Rous sarcoma* virus gene expression, there has been tremendous progress in the understanding and application of antisense oligodeoxy-nucleotides[7]. However in this modern world antisense technology has become an essential laboratory tool to study and understand the functions of any newly discovered genes. This antisense approach should allow the design of drugs that specifically intervene with the expression of any gene whose sequence is known by that it will be more convenient for the treatment for genetic disorders or infections[8]. When the genetic sequence of a particular gene is known to be causative of a particular disease. It is possible to synthesize a strand of nucleic acid (DNA, RNA or a chemical analogue) that will bind to the messenger RNA (mRNA) produced by that gene and inactivate it, effectively turning that gene "off". This is because mRNA has to be single stranded for it to be translated. Alternatively, the strand might be targeted to bind a splicing site on pre-mRNA and modify the exon content of mRNA[9]. Antisense drugs are being researched to treat a variety of diseases[10,11] such as cancers including lung cancer, colorectal carci-noma, pancreatic carcinoma, maligna-ntglioma and malignant melanoma,

diabetes, amyotrophic lateral sclerosis (ALS), duchenne muscular dystrophy and some other diseases such as asthma, arthritis and pouchitis etc,. Most potential therapies have not yet produced significant clinical results, though two antisense drugs have been approved by the U.S. Food and Drug in istration (FDA), fomivirsen (marketed as Vitravene) as a treatment for cytomegalovirus retinitis and mipomersen (marketed as Kynamro) for homozygous familial hypercholesterolemia[12].

Antisense oligonucleotides- chemistry

In general, to target the total pool with in the cells, short and hybridize unique sequence which containing short oligonucleotides (13 -15 nucleotides) were considered[13]. These short nucleotides are out product (as unmodified or chemically modified) of single stranded DNA molecules. This synthesis was direct towards 3'-5' direction. Product such as phosphodiester oligonucleotides which may not be used in antisence experiments due to its unidirectional functions on key proteins which results in cell growth inhibitions. By many chemical modification methylphosphophonates oligonucleotides (First Chemically synthesized) were synthesized by the replacement of the methyl group in the place of non bridging oxygen atom which has an excellent stability in biological system. Even though, due to many limitations, application of phosphodiester oligonucleotides was minimized or better to say as restricted in antisense technology[14]. The phosphorothioates are the most widely studied oligonucleotides, because of their nuclease stability, ease of synthesis. In this particular oligonucleotide non-bridging oxygen atom was replaced by sulphur at each phosphorus group. By that it attains oligonucleotide chain with high solubility and excellent antisense activity[15]. During last two decades, it was clearly evidenced by publishing many data's which stands as a key setup to generate antisense effects in tissues cultures, both in *In vivo and In vitro*, driven latter in to clinical therapeutic trials, naming few among this are G3139, an 18-mer which were targeted as initiation codons of the *bcl*-2 mRNA (now being evaluated clinically in melanoma, chronic lymphocytic leukemia, and other tumors) and ISIS 3521(lung carcinoma)[16]. The main mechanism is known to induce sequence-independent effects attributable to its length dependent high affinity for various cellular proteins, especially heparin-binding growth factors, such as acidic fibroblast growth factor, basic fibroblast growth factor, platelet- derived growth factor, vascular endothelial growth factor, and a host of other heparin-binding molecules, such as laminin, fibronectin, and Mac-1. Further these phosphorothioates will continue to be extensively and exclusively used in the clinical trial settings[17-20]. Peptide nucleic acid (PNA, s) are the another excellent outcome product of basic oligonucleotide modifications. This contains nucleic acid analogous in uncharged, flexible, polyamide back bone which is comprised of repeating N-(2-aminoethyl) glycine units. To this glycine, via methylene carbonyl linker's nucleobases are attached. These oligomers will form very stable duplexes or triplexes with nucleic acids, i.e with double or single-strand DNA or RNA[21,22]. Morpholino oligomer is another compound which has several positive factors to claim as antisense compound. In this deoxyribose moiety is replaced by a morpholine ring and was linked by the uncharged phosphorodiamidate linkage instead of charged phosphor di-ester inter subunit linkages[23]. By this these compounds attains maximum stability over biological system and exhibit efficient antisense activity in cell free translation systems. This system was strengthened by using scrap loading

technique and permeation with streptolysin-O, thus facilitating the oligonuclotide penetration with increases antisense effect[24-27]. Another example of a "second-generation" oligonucleotide is the N3'- P5' phosphoramidate (PN), which result from the replacement of the oxygen at the 3' position on ribose by an amine group[28,29]. These oligonucleotides can, relative to form very stable complexes with RNA and single - or double-stranded DNA and can exhibit highly selective and specific antisense activity *in vitro* and *in vivo*[30]. It clearly demonstrated by the experiment in mice treated with 900µg/day of oligonucleotide were survived for the weeks of 30 were as the control mice treated with mismatched oligonuclotide has survived only for 7 weeks.

Mechanism of action of Antisense compounds

The concept behind antisense technology is quite straightforward: the use of a sequence, complementary by virtue of Watson-Crick base pairs hybridization. These Oligonucleotide are modulated to a specific mRNA which can inhibit its expression and then induce a blockade in the transfer of genetic information from DNA to protein, but mechanism of induction of its biological effect is substle and complex. On basis of mechanism of action oligonucleotides can be discerned in to two types: (a) degradation of mRNA by RNase H-dependent oligonnucloetide; (b) the steric-blocker oligonucleotides, which physically prevent or inhibit the progression of splicing or the translational machinery[31,32].

Delivery of Oligonucleotides

In order for an antisense oligonucleotide to gene expression, it reaches targeted cells effectively. As such to date, the precise mechanisms involved in oligonucleotide penetration is not clear. Uptake is influenced by many factors like active transport, which in turn depends on temperature[33,34], the structure and the concentration of the oligonucleotide[35], and the cell line used. At the present time, it is strongly believed that high concentration of oligonucleotide having influence on its internalization which depends upon the major mechanism of adsorptive endocytosis and fluid phase pinocytosis. In case of low concentration internalization is intiated via interaction with a membrane-bound receptor and to be observed as poor strategies over the effector site.

Need of Developing Delivery systems:

The major limiting step in anti sense compounds application is the inefficient delivery of them to cells and poor bioavailability to intracellular targets due to rapid degradation by nucleases, cellular uptake and subsequent intracellular trafficking. The poly-anionic nature and the large size of antisense compounds render them practically impermeable to cell membranes and consequently their biological activity is significantly compromised. So, it creates an demand to develop novel antisense delivery system towards exits one[36]. Although viral vectors have been widely used to transfer genetic material into cells[37,38] they bear an inherent risk for the patient to encounter severe immunological responses or even develop other deceases[39-42]. As a result of these problems, much attention has been paid in recent years to develop non-viral delivery systems. Among different non-viral systems- cationic polymers, cationic liposomes, polymeric nanoparticles and cell-penetrating peptides (CPPs) represent an attractive concept to bypass the problem of poor membrane permeability of charged macromolecules[43]

Delivery strategies of Oligonucleotides

The aims for optimal delivery of antisense compounds are therefore to

enhance cellular uptake and improved to exit from sub-cellular compartments as correct targeting (spatial and temporal) to a particular site of action. On based on this following are important non viral drug delivery forces and described in brief.

Poly Cations

Polycations and polyanions are poly electrolytes. These groups will dissociate in aqueous solutions (water) and make the polymers charged. Charged molecular chains, commonly present in soft matter systems plays a fundamental role in determining structure, stability and interactions of various molecular assemblies. Poly cationic water-soluble block copolymers consisting of polyoxyethylene (PEO) and polyspermine (PS) chains have been developed for the delivery of antisense oligonucleotides (Figure 2) into the target cells[44]. Cationic polymers constitute one of the most promising approaches to the use of viral vectors for gene therapy[45].

Liposomes

Liposome is vesicular structures consisting of hydrated bilayers. This structures used for pharmaceutical purposes with phospholipids as back bone[46]. Liposomes could also be effective delivery systems for DNA[47, 48] and for nucleic acid-based therapeutics such as antisense oligonucleotides (Figure 3) and siRNA[49]. There are several new methods of liposome preparations are available which are based on lipid drug interaction and liposome disposition. Mechanism involved is inhibition of rapid clearance of liposome by controlling particle size, charge and surface hydration. The liposomes are characterized with respect to physical, chemical and biological parameters. This mode of drug delivery lends more safety and efficacy to administration of several classes of drugs like antiviral, antifungal, antimicrobial, vaccines, anti-tubercular drugs and gene therapeutics. Present applications of the liposome deliveries are in immunology, dermatology, vaccine adjuvant, eye disorders, brain targeting, infective disease and in tumor therapy. The new development in this field is specific binding properties of a drug-carrying liposome to a target cell such as a tumor cell and specific molecules in the body (antibodies, proteins, peptides etc.)[50]. A number of general principles have emerged from the large and rapidly growing literature in the field of nucleic acid delivery51. Liposome's are considered very promising delivery systems for antisense therapeutic approach, offering drug protection and facilitating oligonucleotide cell internalization. Liposomes are the most wide-spread non-viral carriers for nucleic acid delivery. Although anionic and neutral liposomes have been studied for ODN delivery, their poor nucleic acid entrapment efficiency has limited their uses[52].

Cationic Lipids

Writhing Delivery of oligonucleotides and genes to their intracellular targets is a prerequisite for their successful use in medical therapy. Recently a novel group of cationic ampiphiles has afford protection to the nucleic acids has been elucidated[53]. Cationic liposome's are another most commonly used and promising delivery systems for oligonucleotides and genes. These entrap nucleic acids efficiently through formation of complexes which are called lipoplexes[54].Lipid fusion plays an important role in the cationic liposome-mediated delivery of these compounds. Fusion is involved in complex formation between the nucleotides and lipids lays in between extracellular materials with the complexes, as well as in the intracellular trafficking of the delivery system and its load. Since lipid fusion is such a crucial factor in polynucleotide delivery, its controlled use is

important for the success in oligonucleotide and DNA delivery[55-58].

Nanoparticles

In recent years, there has been a rapid increase in nanotechnology in the fields of medicine and more specifically in targeted drug delivery[59].Nanoparticles are also considered to have potential as novel intravascular or cellular probes for both diagnostic and therapeutic purposes (drug/gene delivery), which is expected to generate innovations and play a critical role in medicine. Target-specific drug/gene delivery and early diagnosis in cancer treatment is one of the priority research areas in which nanomedicine will play a vital role. Major classes of nanoparticles include, drug conjugates and complexes, dendrimers, vesicles, micelles, core shell particles, microbubbles, and carbon nanotubes. Most of these formulations have been described as carriers of either drugs or contrast agents (Figure 4)[60]. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. Nanoparticles have been prepared most frequently by three methods: 1. dispersion of preformed polymers, 2.polymerization of monomers and 3.ionic gelation or coacervation of hydrophilic polymers. Other methods includes supercritical fluid technology[61] and particle replication in non-wetting templates (PRINT)[62,63].

Dendrimers:

Dendrimers can act as vectors, in gene therapy, these belong to a family of nanosized, three-dimensional polymers characterized by a unique tree-like branching architecture and compact spherical geometry in solution. Dendrimers are synthesized by a repetitive step-growth polymerization process. Intrinsic viscosity is another important characteristic that distinguishes dendrimers from more conventional polymers[64]. The internal cavity of an appropriately designed dendritic structure could be used for the entrapment of drugs with the possibility of successive controlled release. Studies by several research groups have shown that the interior of a dendrimer is capable of encapsulating guest molecules. The first strategy for the entrapment of guest molecules in dendrimers is physical encapsulation. The second strategy for the encapsulation of guest molecules in dendrimers is based on multiple noncovalent chemical interactions, such as hydrogen bonding, between guest molecules and the dendritic arrangement. Monodispersity, polyvalency, biodegradability and non immunogenicity are the various significant properties for dendrimers which makes them more effective and useful in drug encapsulation[65].

Cell penetrating peptides:

The difficulty of delivering large molecules like proteins, peptides, and nucleic acids into cells through the cell membrane (cellular uptake) has proven a significant impediment to medicinal chemists and the pharmaceutical industry as a whole. The plasma membrane prevents direct translocation of hydrophilic macromolecules by acting as a barrier to efficient and controlled intracellular delivery. Thus, novel efficient carrier delivery methods have to be developed to impart good bioavailability of drug molecules (Figure 5). Peptides which are able to penetrate the cell membrane are known as cell-penetrating peptides (CPPs). They are generally 10 to 30 amino acid residues in length, and either arginine-rich, amphipathic and lysine-rich or hydrophobic[66]. CPPs can be broadly classified as protein derived, chimeric (derived from two or more genes which are coded for separate proteins), or synthetic. CPPs share common features such as positively charged amino acids,

hydrophobicity, and amphipathicity. The discovery of CPPs' ability to traverse the cell membrane opens up a new avenue for drug delivery. Attaching therapeutically significant biomolecules to CPPs provides a means to transport them across the cell membrane. A major breakthrough in the field was the delivery of peptide-nucleic acids (PNAs) using the chimeric peptide transportation[67, 68].

Conclusion

Over the past two decades, the antisense oligonucleotide technology has emerged as a valid approach to selectively modulate gene expression in all the ways and means. By adhering to a strict set of specific rules, ongoing *in vitro* studies using antisense oligonucleotides have given a clear cut way to new targets for potential therapeutic compounds. Apart this, antisense therapy represents a promising and evolving approach to the management of numerous diseases in which a specific abnormality has been identified as a primary or major contributing etiologic factor. The number of *in vitro* experiments has increased continuously, and this has led to numerous therapeutic trials, a few of which now appear preliminarily to be positive. This review highlights the large diversity of particulate systems that have been designed for the delivery of anti sense compounds. There are still many difficulties to be overcome before any of them can proceed into clinical trials. The development of 'smart' nanotechnologies able to control the interactions with biological fluids and to be recognized by target cells should be pursued. The research on alternative routes of administration other than parenteral and transdermal systems should also be extended. However, the optimal usage of antisense oligonucleotides requires the attention towards the effective design, in enhancing its efficacy towards its biological activity and pinpoint delivery. Authors strongly believe that current research towards the antisense therapy will be continued to shed light on ways to increase specificity, increase therapeutic and increase therapeutic efficacy.

Authours Contribution

All authors are equally contributed towards this manuscript in Conception, Design, Manuscript writing, Financial support, Administrative support, Collection of study materials, Data analysis and Interpretation.

Acknowledgement

The author whole heartedly thanks to Seven Hills College of Pharmacy, Tirupathi, for their partial contribution in publishing this article without which this would have not been possible. We thank to BBB solution for their partial contribution in publishing this article without which this would have not been possible.

References

1. Mancuso M, Orsucci D, Filosto M, Simoncini C, Sicilian G. Drugs and mitochondrial diseases: 40 queries and answer. *Expert Opinion Pharmacother*. 2012; 13:527-543.
2. Scherer LJ, Rossi JJ. Approaches for the sequence-specific knockdown of mRNA. *Nat Biotechnol*. 2003; 2:1457-1465.
3. Waga S, Stillman B. The DNA replication fork in eukaryotic cells. *Annu Rev Biochem*. 1998; 67:721-751.
4. Banji D, Banji OJ, Pinnapureddy J. Effacing diseases with Antisense Oligonucleotide. Der Pharma Lett. 2010; 2: 393-407.
5. Dias N, Stein CA. Antisense oligonu-cleotides: basic concepts and mechanisms. Mol. Cancer Ther. 2002; 1:347-355.
6. Pirollo KK, Rait A, Sleer LS, Chang EH. Antisense therapeutics: from theory to clinical practice. *Pharmacol Therapeut*. 2002; 99; 55– 77.
7. Zamecnik PC, Stephenson M. Inhibition of Rous sarcoma virus replication and cell transformation by a specific

oligodeoxynucleotides. *Proc Natl Acad Sci. USA*.1978; 75: 280–284.
8. Kurreck J. Antisense technologies. *Eur J Biochem*.2003; 270: 1628-1644.
9. Morcos PA, Achieving targeted and quantifiable alteration of mRNA splicing with Morpholino oligos. *Biochem Biophys Res Commun*.2007; 358: 521–7.
10. Gallego J, Varani G. Targeting RNA with small-molecule drugs: therapeutic promise and chemical challenge. Accounts Chem Res.2001; 34: 836-843.
11. Bayer TS, Smolke CD. Programmable ligand-controlled riboregulators of *eukar yotic gene expression. Nat Biotechnol*. 2005; 23: 337-343.
12. Weiss B, Davidkova G, Zhou LW. Antisense RNA gene therapy for studying and modulating biological processes. *Cell Mol Life Sci*.1999; 55: 334-358.
13. Eder PS, DeVine RJ, Dagle JM, Walder JA. Substrate specificity and kinetics of degradation of antisense oligonucleotides by a 3′ exonuclease in plasma. *Antisense Res Dev*.1991; 1: 141–151.
14. Goodchild J. Conjugates of oligonucleotides and modified oligonucleotides: a review of their synthesis and properties. Bioconjugate Chem.1990; 1: 165-187.
15. Jansen B, Wacheck V, Heere-Ress E, Schlagbauer-Wadl H, Hoeller C, Lucas T, Hoermann M, Hollenstein U, Wolff K, Pehamberger H, Chemosensitization of malignant melanoma by BCL2 antisense therapy.Lancet.2000;356:1728–1733.
16. Agrawal S, Zhao Q. Antisense therapeutics. Curr. Opin. Chem. Biol., 1998, 2, 519-528.
17. Lebedeva I, Benimetskaya L, Stein C,Vilenchik M. Cellular delivery of antisense oligonucleotides. *Eur J Pharm Biopharm*.2000; 50: 101-119.
18. Scherer LJ, Rossi JJ. Approaches for the sequence-specific knock down of mRNA. *Nat Biotechnol*. 2003; 21: 1457-1465.
19. Guvakova MA, Yakubov LA,Vlodavsky I,Tonkinson JL, Stein CA. Phosphorothioate oligodeoxynucleotides bind to basic fibroblast growth factor, inhibit its binding to cell surface receptors, and remove it from low affinity binding sites on extracellular matrix. *J Biol Chem*.1995; 270: 2620–2627.
20. Fennewald SM, Rando RF. Inhibition of high affinity basic fibroblast growth factor binding by oligonucleotides. *J Biol Chem*.1995; 270:21718–21721.
21. Egholm M, Buchardt O, Christensen L, Behrens C, Freier SM, Driver DA, Berg RH, Kim SK, Norden B, Nielsen PE. PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. Nature. 1993; 365: 566–568.
22. Nielsen PE, Egholm M, Berg RH, Buchardt. Sequenceselective recognition of DNA by strand displacement with a thyminesubstituted polyamide. Science.1991; 254: 1497–1500.
23. Summerton J,Weller D. Morpholino antisense oligomers: design, preparation, and properties. *Antisense Nucleic Acid Drug Dev*.1997;7;187–195.
24. Hudziak RM, Summerton J, Weller DD, Iversen PL. Antiproliferative effects of steric blocking phosphorodiamidate morpholino antisense agents directed against c-myc, *Antisense Nucleic Acid Drug Dev*.2000;10 :163-176.
25. Taylor MF, Weller DD, Kobzik L. Effect of TNF- α antisense oligomers on cytokine production by primary murine alveolar macrophages. *Antisense Nucleic Acid Drug Dev*. 1998; 8: 199–205.
26. Summerton J, Stein D, Huang SB, Matthews P, Weller D M , Partridge M, Morpholino and phosphorothioate antisense oligomers compared in cell-free and in-cell systems. *Antisense Nucleic Acid Drug Dev*.1997; 7: 63–70.
27. Giles RV, Spiller DG, Clark RE, Tidd, DM. Antisense morpholino oligonucleotide ana-log induces missplicing of C-myc Mrna. *Antisense Nucleic Acid Drug Dev*.1999;9: 213–220.
28. Gryaznov SM, Lloyd DH, Chen JK, Schultz RG, DeDionisio LA, Ratmeyer L and Wilson WD. Oligonucleotide N3' --> P5' phosphoramidates. *Proc Natl Acad Sci USA*., 1995; 92: 5798-5802.
29. Chen JK,Schultz RG, Lioyd DH, Gryaznov SM. Synthesis of oligodeoxyribonucleotide N3′→ P5′ phosphoramidates. *Nucleic Acids Res*.1995; 23: 2661-2668.

30. Skorski T, Perrotti D, Nieborowska-Skorska M, Gryaznov S, Calabretta B. Antileukemia effect of c-myc N3'- P5'phosphoramidate antisense oligonucleotides *in vivo*. *Proc Natl Acad Sci USA*.1997; 9: 3966–3971.
31. Dean NM, McKay R, Condon TP, Bennett CF. Inhibition of protein kinase C-_ expression in human A549 cells by antisense oligonucleotides inhibits induction of intercellular adhesion molecule 1 (ICAM-1) mRNA by phorbol ester. *J Biol Chem*.1994; 269: 16416-16424.
32. Larrouy B, Blonsk C, Boiziau C, Stuer M, Moreau S, Shire D, Toulme JJ. RNase H-mediated inhibition of translation by antisense oligodeoxyribonucleotides: use of backbone modification to improve specificity. Gene.1992; 121: 189–194.
33. Loke SL, Stein CA, Zhang XH, Mori K, Nakanishi M, Subasinghe C, Cohen JS, Neckers LM. Characterization of oligonucleotide transport into living cells. *Proc Natl Acad Sci USA*.1989; 86: 3474–3478.
34. Yakubov LA, Deeva EA, Zarytova VF, E.M. Ivanova EM, Ryte AS, Yurchenko LV Vlassov VV. Mechanism of Oligonucleotide uptake by cells: involvement of specific receptors. *Proc Natl Acad Sci USA*. 1989; 86: 6454–6458.
35. Vlassov VV, Balakireva LA, Yakubov LA. Transport of oligonucleotides across natural and model membranes. *Biochim Biophys Acta*.1994; 1197: 95-108.
36. Akhtar S, Hughes MD, Khan A, Bibby M, Hussain M, Nawaz Q, Double J, Sayyed P. The delivery of antisense therapeutics. *Adv Drug Deliver Rev*.2003; 44: 3-21.
37. Kootstra NA, Verma IM. Gene therapy with viral vectors. *Annu Rev Pharmacol. Toxicol*.2003; 43: 413-439.
38. Verma IM, Weitzman MD. Gene therapy: twenty-first century medicine. *Annu Rev Biochem*. 2005; 74: 711-738.
39. Raper SE, Yudkoff M, Chirmule N, Gao GP, Nunes F, Haskal ZJ. A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. Hum. Gene ther.2002; 13: 163-175.
40. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N. Leboulch P. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science.2003; 302: 415-419.
41. Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab*. 2003; 80: 148-158.
42. Check E. Gene therapy put on hold as third child develops cancer. Nature. 2005; 433: 555-561.
43. Laufer SD, Restle T. Peptide-mediated cellular delivery of oligonucleotide based therapeutics *in vitro*: quantitative evaluation of overall efficacy employing easy to handle reporter systems. *Curr Pharm Design*. 2008; 14: 3637-3655.
44. Safinya CR. Structures of lipid–DNA complexes: supramolecular assembly and gene delivery. *Curr Opin Struc Biol*. 2001; 11: 440-448.
45. Tiera MJ, Shi Q, Winnik FM, Fernandes JC. Polycation-based gene therapy: current knowledge and new perspectives. *Curr Gene Ther*.2011; 11: 288-306.
46. Kulkarni PR, Yadav JD, Vaidya KA. Liposomes: A Novel Drug Delivery System. *Int J Curr Pharm Res*.2011; 3: 10-18.
47. Fraley R, Subramani S, Berg P, Papahadjopoulos D. Introduction of liposome encapsulated SV40 DNA into cells. *J Biol Chem*.1980; 255:10431–10435.
48. Lian T, Ho RJ. Trends and developments in liposome drug delivery systems. J Pharm Sci.2001; 90: 667-680.
49. Li W, Szoka Jr FC. Lipid-based nanoparticles for *nucleic acid delivery. Pharm Res*.2007; 24: 438-449.
50. Samad Y, Sultana, Aqil M. Liposomal drug delivery systems: an update review. *Curr Drug Deliv*.2007; 4: 297-305.
51. Stuart DD and Allen TM. A new liposomal formulation for antisense oligodeoxynucleotides with small size, high incorporation efficiency and good stability. *Biochim Biophys Acta*. 2000; 1463: 219–229.

52. Ruozi B, Battini R, Tosi G, Forni F, Vandelli MA. Liposome oligonucleotides interaction for *in vitro* uptake by COS I and HaCaT cells. *J Drug Target*.2005; 13: 295-304.
53. DeLong RK, Yoo H, Alahari SK, Fisher M, Short SM, Kang SH, Kole R, Janout V, Regan SL, Juliano RL. Novel cationic amphiphiles as delivery agents for antisense oligonucleotides. *Nucleic Acids Res*.1999; 27: 3334–3341.
54. Templeton NS, Lasic DD, Frederik PM, Strey HH, Roberts DD, Pavlakis GN. "Improved DNA: liposome complexes for increased systemic delivery and gene expression. *Nat Biotechnol*.1997; 15: 647-652.
55. Maurer N, Wong KF, Stark H, Louie L, Mcintosh D, Wong T, Scherrer P, Semple SC, Cullis PR. Spontaneous entrapment of polynucleotides upon electrostatic interaction with ethanol-destabilized cationic liposome. *Biophys J*.2001; 80: 2310-2326.
56. Bailey AL, Sullivan SM. Efficient encapsulation of DNA plasmids in small neutral liposomes induced by ethanol and calcium. *Biochim Biophys Acta*.2000; 1468: 44-54.
57. Semple SC, Klimuk SK, Harasym TO, Santos ND, Ansell SM, Wong KF, Maurer N, Stark H, Cullis PR, Hope MJ, Scherrer P. Efficient encapsulation of antisense oligonucleotides in lipid vesicles using ionizable aminolipids: formation of novel small multilamellar vesicle structures. *Biochim Biophys Acta*.2001; 1510: 152-166.
58. Monkkonen J, Urtti A. Lipid fusion in oligonucleotide and gene delivery with cationic lipids. *Adv Drug Deliv Rev*.1998; 34: 37-49.
59. Sahoo SK, Labhasetwar V. Nanotech approaches to drug delivery and imaging. *Drug Discov Today*.2003; 8: 1112-1120.
60. Janib SM, Moses AS, MacKay JA. Imaging and drug delivery using theranostic Nanoparticles. *Adv Drug Deliv Rev*.2010; 62: 1052-1063.
61. Reverchon E, Adami R. Nanomaterials and supercritical fluids. *J Supercrit Fluids*. 2006; 37:1-22.
62. Rolland JP, Maynor BW, Euliss LE, Exner AE, Denison GM, DeSimone J. Direct fabrication and harvesting of monodisperse,shape-specific nano biomaterials. *J Am Chem Soc*.2005; 127: 10096-10100.
63. Mohanraj VJ, Chen Y. Nanoparticles-a review. *Trop J Pharm Res*.2007; 5: 561-573.
64. Aulenta F, Hayes W, Rannard S. Dendrimers: a new class of nanoscopic containers and delivery devices. *Eur Polym J*.2003; 39: 1741-1771.
65. Garg T, Singh O, Arora S, Murthy RSR. Dendrimer-a novel scaffold for drug delivery. *Int. J.Pharm. Sci. Res*., 2011, 7, 211-220.
66. Chugh A, Eudes F, Shim YS. Cell-penetrating peptides: Nanocarrier for macromolecule delivery in living cells. *Int Union Biochem Mol Biol*.2010; 62: 183-193.
67. Heitz F, Morris MC, Divita G. Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. *Br J Pharmacol*. 2009; 157: 195-206.
68. El-Andaloussi S, Holm T, Langel U. Cell-penetrating peptides: mechanisms and applications. *Curr Pharm Design*.2005; 11: 3597-3611.

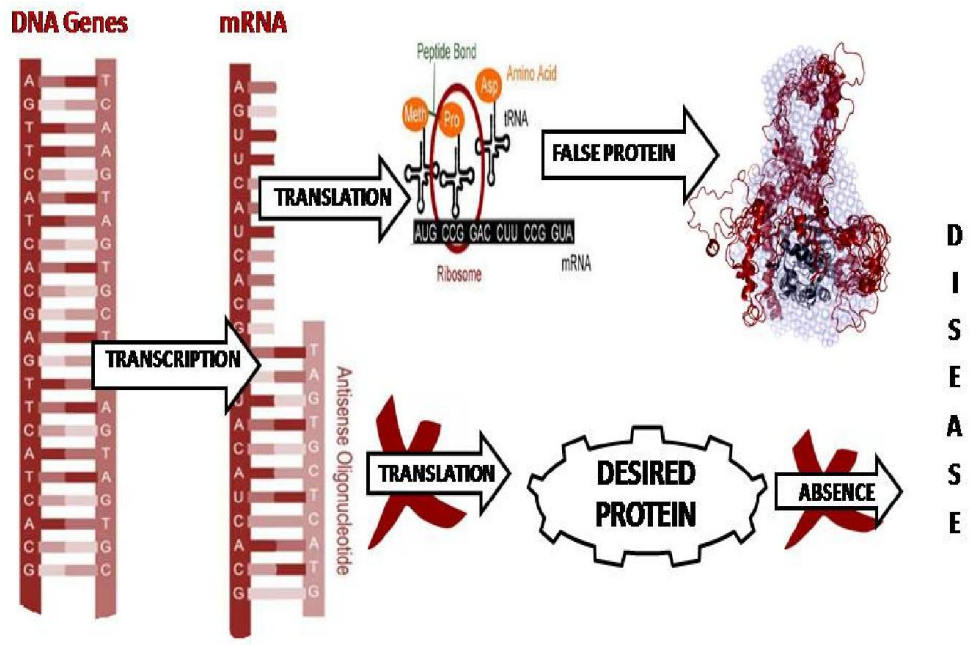

Fig. 1 Basis concepts in antisense technology in protein modification.

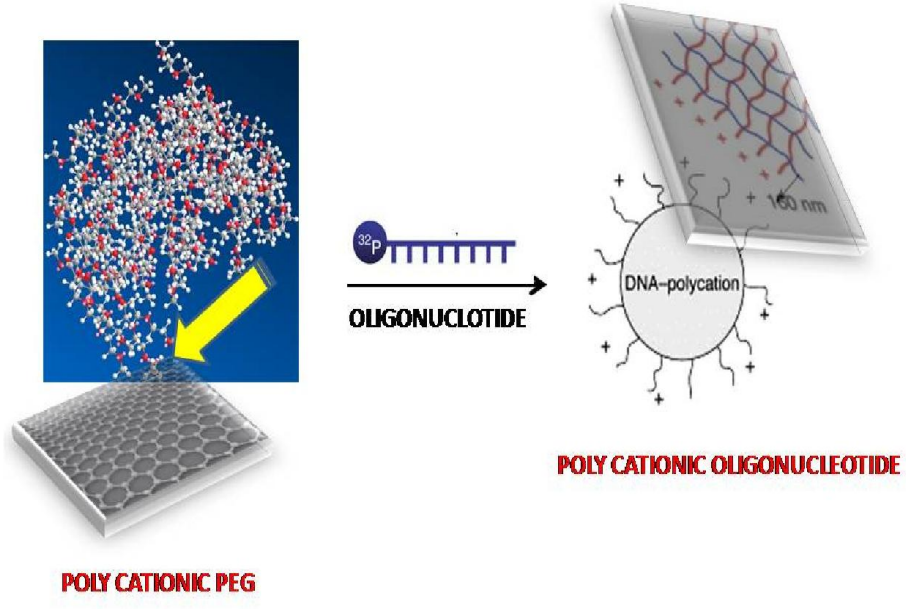

Fig. 2 Mechanism involved in cross-linked polycation and PEG network with

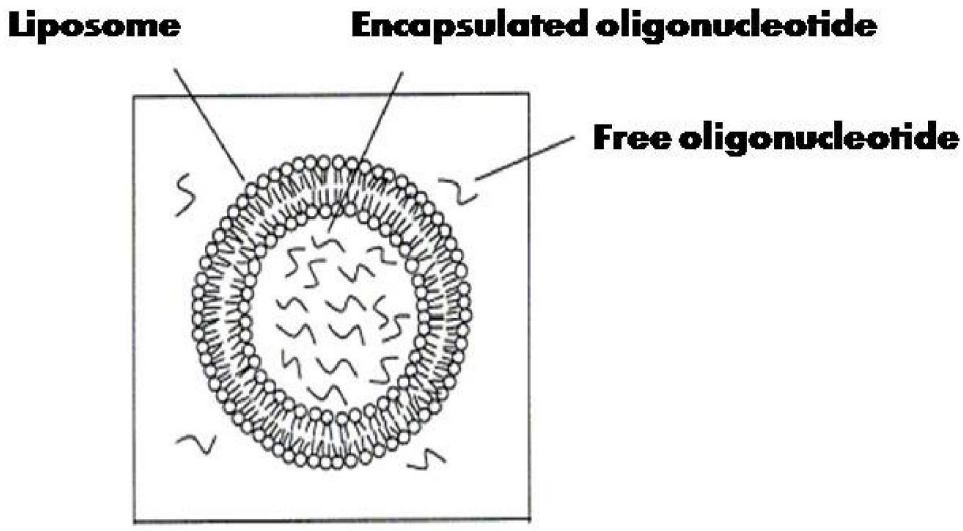

Fig. 3 Encapsulated Oligonucleotide with liposomes.

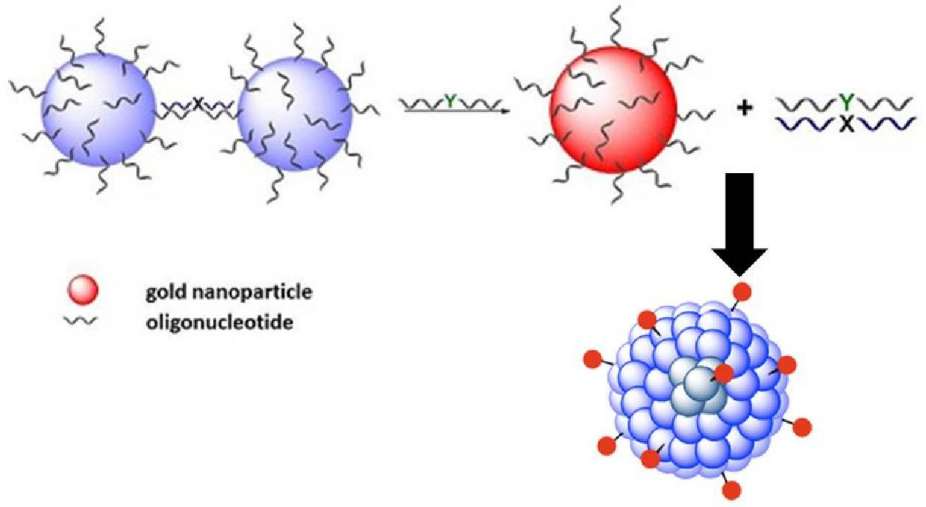

Fig.4 -Chemically modified oligonucleotides and the development of Nanoparticles-linked oligonucleotide probes.

 ISSN-2347-5447

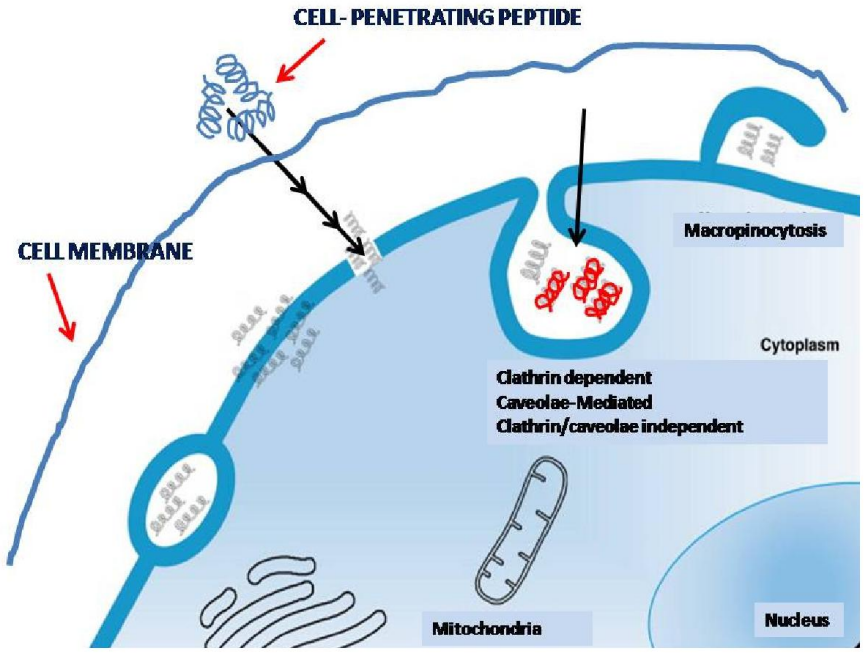

Fig.5 -Oligonucleotide penetration mechanism through cell membrane and its release mechanism in cytoplasm.