Editorial

An overview of viral and nonviral delivery systems for microRNA

MicroRNAs (miRNAs) are small endogenous noncoding RNAs that direct posttranscriptional regulation of gene expression. Mature miRNAs function as components of RNA-induced silencing complex and interact directly with the 3'-untranslated region of the target mRNA. The target mRNA is then cleaved when the full complementarity of miRNA-mRNA is achieved. For partial complementarity, the translation of the target mRNA is repressed. It has been estimated that about one-third of human genes are regulated by miRNAs.[1] Deregulation of miRNAs has been found in many diseases including cancer, neurologic disease, metabolic disorders, and cardiovascular disease.

Due to the important function of miRNAs, miRNA therapy has attracted significant attention. miRNA therapy could be categorized into two mechanisms, according to deregulation status of the target mRNA: miRNA replacement therapy that restores the miRNA expression by transduction of exogenous miRNA, and miRNA inhibition therapy that inhibits the miRNA expression by designing anti-miRNA oligonucleotides known as antagomirs.[2,3] Currently, both viral and nonviral miRNA delivery systems are used, and there are advantages and disadvantages for each approach.

Viral-based systems usually use retroviruses, lentiviruses, and adenoviruses or adeno-associated viruses (AVV) as delivery vectors.[4] These viral vectors are modified in some specific genomic area so that they are unable to replicate, and their safety are increased. The advantage of this delivery system was to provide high transfection or infection efficiency, and a high level of constant expression of miRNAs or antagomirs.

Retroviral vectors are frequently employed to deliver miRNAs into somatic and germline cells. Retroviruses belong to RNA virus family with a genome size of about 7-11 KB.[5] The retroviral genes are removed in the constructed vectors so that space up to 8 KB is made for foreign genetic sequences. Since, the genetic materials carried by the retroviral vectors are integrated into host cell genomic DNAs during the mitotic phase of cell cycle; these vectors are utilized for infecting dividing cells only. Integration of exogenous sequence into host genome enables the stable expression of miRNA. The miRNA expression might be increased greatly by retroviral infection. For example, miR-138 was induced about more than 1,000-fold in mouse embryonic fibroblasts in order to enhance the production of pluripotent stem cells.[6] The miRNA inhibition therapy is also very efficient in the retroviral system. The miR-205 was almost completely repressed in the skin stem cells.[7]

Lentiviruses are a subgroup of retroviruses, which integrate foreign genetic material into the host genome. Similar to retroviral vectors, lentiviral vectors are able to transfer about 8 KB genetic sequences into host cells. In contrast to retroviruses, lentiviruses can infect both dividing and nondividing cells due to their pathogenic characteristics.[8] Therefore, lentiviral vectors are frequently employed to infect postmitotic and terminal differentiated cells to treat neurologic disorders. The lentiviral system produces high transfection efficiency and long-term stable expression of introduced miRNAs. A study found that miR-143 was increased about 2,500-fold in corneal epithelial progenitor cells.[9]

Adenoviruses are double-stranded DNA viruses and contain more than 100 serotypes. Compared to retroviral vectors, adenoviral systems can transfer up to 38 KB foreign DNA, but are unable to integrate the exogenous sequences into host genomic DNA. In contrast to adenoviruses, AAVs are more frequently utilized in miRNA gene delivery. AAVs are single-stranded DNA viruses, which consist of 12 primate serotypes (AAV1-AAV12). AAV vectors could only accommodate up to 4.8 KB exogenous genetic material, which limits its utilization in large gene delivery. However, AAV vectors are favorable in transferring miRNAs due to the small size of miRNA genes.[10] Similar to lentiviruses, adenoviruses, and AAVs can transfect both dividing and nondividing cells. The miR-375 was increased about 17-fold in alveolar epithelial cells using an adenoviral vector.[11] To induce cardiac regeneration, miR-590 and miR-199a were delivered into neonatal mouse heart by an rAAV9 vector. It was found that miR-590 was induced about 240-fold, and miR-199a was increased 3.5-fold compared to the empty rAAV9 vectors.[12]

Access this article online	
Quick Response Code:	Website: www.jpionline.org
	DOI: 10.4103/2230-973X.167646

How to cite this article: Yang N. An overview of viral and nonviral delivery systems for microRNA. Int J Pharma Investig 2015;5:179-81.

Although, the viral vectors are replication-deficient, there are some problems that restrict their usages such as toxicity due to the production of toxin, immunogenicity that induce inflammatory response and tissue degeneration, and mutations caused by the inserted sequence. To avoid these limitations, nonviral delivery systems are widely used.

In contrast to viral delivery systems, nonviral systems are much less toxic, immunogenic, and no limitation of the size on the transferred DNA, but lower transfection efficiency. Effective nonviral delivery systems should transport the exogenous miRNAs or miRNA-expressing vectors, and protect these sequences from cellular nuclease-mediated degradation nonviral delivery systems consist of physical approaches and chemical approaches. Physical approaches exert external forces to make the cell membrane transient permeable for the gene delivery, which include gene gun, electroporation, hydrodynamic, ultrasound, and laser-based energy. Physical methods might damage the cell integrity and cause high apoptotic rate, and are hard to prevent nuclease cleavage. Physical approaches are usually applied *in vitro* studies and seldom used in the miRNA delivery.[4] Chemical methods consist of lipid-based, polymer-based, and inorganic carriers.[3,13]

Lipid-based approaches utilize the lipid/nucleic acid complexes, named lipoplexes or liposome as delivery carriers. Liposomes are composed of the membrane-like surface, and nucleic acids encapsulated inside. There are three types of liposomes based on the charges: Cationic, anionic, or neutral. Cationic lipoplexes are most commonly used in nonviral delivery systems due to their unique characteristics including ease of production, high affinity with the cell membrane, nonpathogenic, and nonimmunogenic response. Systemic delivery of miR-29b using cationic lipoplex (DOTMA: Cholesterol: TPGS) into nonsmall cell lung cancer cells increased the expression of miR-29b by 5-fold and decreased the tumor growth rate by about 60%.[14] There are many commercially available cationic lipoplex for miRNA delivery, which have been reported to achieve favorable results, such as Lipofectamine® RNAi-MAX (Invitrogen),[15] SilentFect™ (Bio-Rad),[16] DharmaFECT® (Dharmacon),[17] and SiPORT™ (Invitrogen).[18]

The most critical disadvantage of liposome delivery system is the short half-lives (several hours) of the nanoparticles in sera due to nonspecific binding to serum proteins. To avoid the instability caused by the interaction of cationic lipids with serum proteins, anionic and neutral liposomes are sometimes used in miRNA delivery, such as miR-29b delivery in acute myeloid leukemia by anionic lipoplexes,[19] and miR-34a transfer in lymphoma through neutral lipids.[20] Conjugation of the lipids with hydrophilic and flexible polyethylene glycol (PEG) could greatly increase their stability and result in long half-lives (up to 72 h in sera).[21] Pramanik *et al*. constructed lipid nanoparticle by using DOTAP: Cholesterol: DSPE-PEG-OMe at a 1:1:0.2 ratio to deliver miR-143/145 cluster and miR-34a into pancreatic cancer xenograft mice and found increased accumulation of nanoparticles in tumor tissues and decreased tumor size.[22]

Polymer-based approaches utilize polyethylenimine (PEI), poly (lactide-co-glycolide) (PLGA), poly (amidoamine) (PAMAMs) dendrimers, or cell-penetrating peptide (CPP) as delivery carriers. Compared to high molecular weight PEIs and low molecular weight PEIs show lower levels of damage to cell membranes and less cytotoxic to cells. A study used low molecular weight PEIs to deliver miR-33a mimics and miR-145 into colon cancer xenograft mice, resulting in decreased tumor growth and increased cell death.[23] However, the transfection efficiency is really low and PEIs are poorly biodegradable. PLGA is Food and Drug Administration-approved biodegradable copolyester for drug delivery and used in some miRNA therapy studies like delivery anti-miRNAs.[24] The hydrophobic nature of PLGA decreases the miRNA delivery effectiveness. PAMAMs are positively charged polymers and have high transfection efficiency compared to other polymers. PAMAM dendrimer — anti-miR-21 complexes decreased the growth of glioblastoma cells.[25] The major disadvantage of PAMAM dendrimers is the accumulation of the polymers in the liver.[26] Besides these synthetic polymers discussed above, natural derived polymers like CPPs are used for miRNA carriers. For example, an arginine-rich CPP from natural protamine effectively transferred miR-29b into osteogenic stem cells.[27] In contrast to synthetic polymers, CPPs are less toxic but prone to be degraded in sera.

In contrast to lipids and polymers, few studies utilize inorganic materials as miRNA gene delivery. Current inorganic miRNA vectors include gold nanoparticles (AuNPs), Fe_3O_4-based nanoparticles,[28] and silica-based nanoparticles[29] among which GNPs are most frequently used. AuNPs have been reported to successfully deliver miR-130b[30] and anti-miR29b[31] into tumor cells. The inorganic carriers have high stability *in vivo* and are free of microbial attack. However, the interactions between the carriers and nucleic acids are weak. To solve this problem, inorganic and organic hybrid materials are tried. For example, $AuNP_{10}$ was conjugated with $PEG_{0.5}$ to deliver miR-1 into cancer cells and showed higher transfection efficiency, lower toxicity, and longer half-lives compared to lipofection.[32]

Viral and nonviral miRNA delivery systems have both advantages and disadvantages: Viral vectors produce higher transfection efficiency but are more toxic and immunogenic, whereas nonviral carriers have lower delivery efficiency but are much safer. Therefore, effective and safe miRNA delivery systems are in urgent need. The design of future delivery systems should combine the advantages of both systems to employ more miRNA therapies from bench to bedside.

Ningning Yang

Department of Pharmaceutical Sciences, Manchester University College of Pharmacy, Fort Wayne, IN 46845, USA

Address for correspondence: Dr. Ningning Yang, School of Pharmacy, Erie College of Osteopathic Medicine - Bradenton, 5000 Lakewood Ranch Blvd, Bradenton, FL 34211, USA.
E-mail: ningningyoung@gmail.com

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