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Modulating the Efficacy of Small Interfering RNA by Cell Entry Mechanism of Lipid-Based Nanoparticles

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Abstract

Small interfering RNAs (siRNAs) are a new class of medicine that shows the promising potential for diseases treats. However, due to degradation by endonucleases, large size and anionic charge, siRNA delivery is challenging and thus, delivery system is crucial. Lipid-based nanoparticles are mostly used for siRNA delivery. Since siRNAs in lipid-based nanoparticles are usually surrounded by a thin lipid bilayer, the availability of siRNA in cell cytosol is mainly dependent on the endocytosis mechanisms by which complex siRNA lipid-based nanoparticles enter into cells and that mechanisms dictate their intracellular fates, release and efficacy of siRNAs. This mini review provides a brief overview of mechanism of siRNA action, and the cellular entry mechanisms of lipid-based nanoparticles encapsulated with siRNAs and the perspectives for the rational design of nanoparticles in maximizing the efficacy of siRNA drugs.

Keywords: siRNA Drugs; Complex siRNA Lipid-Based Nanoparticles; Endocytosis Pathways; CME; CvME; Endo-Lysosomes; siRNA Efficacy

Introduction

Over 20 years ago, Fire and Mello discovered that small double-stranded RNAs (siRNAs) were capable of selectively causing the targeted gene silencing by degrading its messenger RNA (mRNA) [1,2]. Based on their original reports, there are currently three siRNA drugs, namely patisiran, givosiran and lumasiran in the pharmaceutical market and seven siRNA drugs are in late stages of Phase 3 clinical trials [3]. Lipid-based nanoparticles (LBNPs) are an integral part for the clinical success of siRNA drugs because siRNAs themselves limit the ability of siRNAs to enter into cells owing to their specific properties, including high molecular weight, anionic charge, hydrophilicity, and potential for degradation by nucleases [4].

Lipid-based nanoparticles, including lipoplexes, lipopolyplexes, stable nucleic acid lipid particles and membrane/core nanoparticles are most commonly used for siRNAs delivery [5]. LBNPs can incorporate or complex with siRNAs and help them to get access into the target compartments within cells. Complex siRNA-LBNPs enter cells via endocytosis pathways depending on their physicochemical properties, surface modifications or cell types [5]. Each endocytosis pathway has a distinct pattern of intracellular fate for complex siRNA-LBNPs and thus, the same complex differs in its therapeutic efficiency in various cell types. In general, to obtain siRNA efficacy, after entrance into cells through endocytosis pathways, Complex siRNA-LBNPs must avoid endo-lysosomal degradation and release siRNA into cell cytoplasm and then the cytosolic siRNAs bind to its complementary target sequence causing the targeted gene silencing by degrading mRNAs [4,6].

In this review, we will attempt to provide several endocytosis mechanisms for complex siRNA-LBNPs uptake and their

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corresponding intracellular fates. In addition, we will provide future perspectives for modulating these pathways towards the improvement of the siRNA delivery efficacy against diseases.

Mechanism of siRNA Action

A siRNA is a short double stranded exogenous RNA molecule (approximately 21-23 nucleotide-long sequences) that exhibits its effects at the post-transcriptional level [7]. The endogenous process of siRNA starts in the cell cytosol, when double stranded RNA (dsRNA) is cleaved by an endoribonuclease, Dicer, following to the formation of duplex that is composed of a passenger strand (sense strand) and a guide strand (antisense strand). siRNA gets incorporated with the RNA-induced silencing complex (RISC) and Argouate 2 component, causing the unwinding of duplex which in turn, degrades the passenger strand. Complementary binding of the guide strand to the target mRNA triggers the cleavage of the target sequence which is degraded within cells [8]. In such a way, siRNA inhibits the target gene sequence which is responsible for causing the disease, as shown in Figure 1.

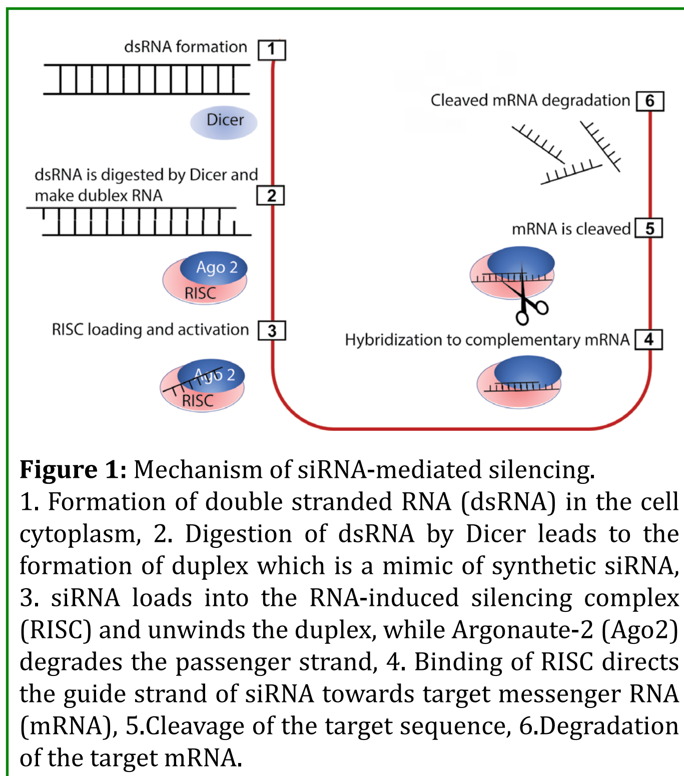

Figure 1: Mechanism of siRNA-mediated silencing.
1. Formation of double stranded RNA (dsRNA) in the cell cytoplasm, 2. Digestion of dsRNA by Dicer leads to the formation of duplex which is a mimic of synthetic siRNA, 3. siRNA loads into the RNA-induced silencing complex (RISC) and unwinds the duplex, while Argonaute-2 (Ago2) degrades the passenger strand, 4. Binding of RISC directs the guide strand of siRNA towards target messenger RNA (mRNA), 5.Cleavage of the target sequence, 6.Degradation of the target mRNA.

Cellular Entry Mechanisms for Complex siRNA-Lipid Based Nanoparticles

As discussed above, the critical step for siRNA activity is to reach siRNAs into the cell cytoplasm because the machinery for the targeted gene silencing mediated by siRNA is located there. Lipid-based nanoparticles are continuously mitigated this demand by delivery siRNAs into cell cytosol [3]. Complex siRNA-LBNPs are mainly taken up by cells through endocytosis pathways, including clathrin-mediated endocytosis (CME), caveolae-mediated endocytosis (CvME) and macropinocytosis [9,10] (Figure 2). Since complex siRNA-LBNPs are mainly internalized into cells by these endocytosis pathways, the study on the internalization pathways would help one to the rational design of complex siRNA-LBNPs through the determination of the intracellular fate of complex siRNA-LBNPs.

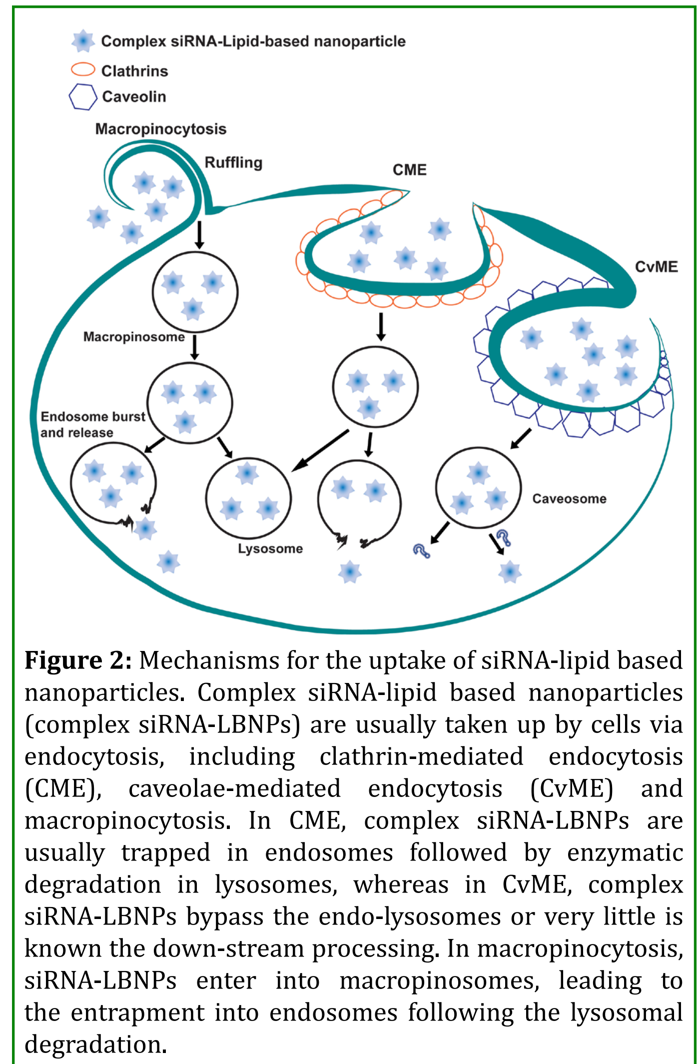

Figure 2: Mechanisms for the uptake of siRNA-lipid based nanoparticles. Complex siRNA-lipid based nanoparticles (complex siRNA-LBNPs) are usually taken up by cells via endocytosis, including clathrin-mediated endocytosis (CME), caveolae-mediated endocytosis (CvME) and macropinocytosis. In CME, complex siRNA-LBNPs are usually trapped in endosomes followed by enzymatic degradation in lysosomes, whereas in CvME, complex siRNA-LBNPs bypass the endo-lysosomes or very little is known the down-stream processing. In macropinocytosis, siRNA-LBNPs enter into macropinosomes, leading to the entrapment into endosomes following the lysosomal degradation.

Clathrin-Mediated Endocytosis (CME)

CME is one of the major and well-characterized endocytic pathways. It is found in all mammalian cells and both low-density lipoprotein and transferrin are typically internalized by CME [11]. In general, CME starts when the strong binding of a ligand and cell surface receptor is occurred. The binding leads to the assembling of clathrins in the polyhedral lattice right on the cytosolic surface of the cell membrane and

then the deformation of membrane helps to the formation of a clathrin-coated pit with a size about 100–150 nm. For this process, GTPase dynamin is crucial. As the lattice is continuously forming, the pit becomes deeply invaginated and fuse with endosomes. The endocytosed vesicles move forward, integrate into late endosomes and finally transport the cargos to either lysosomes or cytosol [12]. Since complex siRNA-LBNPs that are internalized through CME are usually trapped in endosomes followed by enzymatic degradation in lysosomes, and thus, very limited number of siRNAs may be in the cell cytosol for silencing the target mRNAs. Therefore, for siRNAs, it is crucial to avoid lysosomal degradation and release into the cytosol. Several strategies have been developed to increase the cytosolic release of endocytosed particles. This involves the association of vesicular destructive elements to complex siRNA-LBNPs, which can perturb the integrity of the vesicular membrane and allow the cytosolic release of siRNAs, while those are not harmful for the siRNAs. Some cationic polymers, e.g., polyethyleneimine (PEI), and some lipids also have the ability to enhance the cytosolic release of siRNAs through different mechanisms [13-15].

Caveolae-Mediated Endocytosis (CvME)

During CvME, a special flask-shaped structure is initially formed on the cell membrane that is called as caveolae. They are rich in cholesterol and glycosphingolipids. CvME occurs in many cell types but usually are abundant in endothelial cells. The size of caveolae is usually 50–100 nm in diameter and the fission of the caveolae from the membrane is mediated by the GTPase dynamin, which locates in the neck of caveolae and then generates the cytosolic caveolar vesicle [11]. Some receptors such as insulin receptor and epidermal growth factor receptor that are present in caveolae, can mediate CvME [16,17]. CvME is a nonacidic and nondigestive route of internalization; thus, the intracellular fate of particles is different from CME. Most of particles (e.g., SV40 virus) that are internalized by caveolae can be directly transported to the Golgi and/or endoplasmic reticulum, thus avoiding normal lysosomal degradation [18]. We have recently found that siRNA-cationic liposomes when modified with a small amount of gold nanoparticles shifted the uptake pathway from multiple to CvME, avoiding the lysosomal degradation and improved siRNA silencing efficacy. Therefore, it is a promising strategy for complex siRNA-LBNPs delivery by which the internalization can be enhanced and the maximum amount of siRNAs delivery at the cell cytosol might be possible through the use of specific receptors for caveolae or modifications with other nanoparticles.

Macropinocytosis

Macropinocytosis forms large endocytic vesicles of irregular size and shape that are generated by actin-driven evagination of the plasma membrane [19]. Through this process, cells nonselectively take up a large amount of fluid-phase contents when massive fluid-phase endocytosis is necessary. Macropinocytosis is a signal dependent process that normally occurs when macrophages or cancer cells are stimulated by growth factors or others. It is almost similar to phagocytosis and occurs constitutively in antigen-presenting cells. The macropinosomes vary in size, sometimes being as large as 5.0 μm in diameter, and have no apparent coat structures. During this process, both small GTPase and Ras-related in brain (Rab) proteins play an important role for the vesicle fission from the cell membrane [20]. However, there is no significant evidence that explain the relationship between the macropinocytosis and lysosome.

Several reports demonstrated that macropinocytosis is involved in the uptake of both cationic arginine rich peptides (such as TAT and octa arginine (R8)) and its-associated cargos [11]. Both peptides when incorporated with complex siRNA-LBNPs, the combined carriers increased the uptake of carrier, the avoidance of lysosomal degradation and finally enhanced the therapeutic efficacy. In addition, Lee et al. used a PTD called Hph-1 to conjugate vector PEI to deliver siRNA. The result showed that the complexes entered the cells through the non-endocytic pathway, which has a quicker dynamic behavior compared with the endocytosis pathways and is energy-independent because it has high transfection efficiency even in low temperature [21]. However, more and more evidences and more efforts are needed to have a comprehensive understanding of these pathways for the improvement of complex siRNA-LBNPs.

Future Perspectives for Modulation of Uptake Mechanisms of Complex siRNA-LBNPs for Enhancing the Efficacy of siRNAs

There are mainly three types of uptake mechanisms by which complex siRNA-LBNPs, including lipoplexes, SNALPs, lipopolyplexes, and membrane/core nanoparticles can be taken up by cells [5]. Understanding these mechanisms (CME, CvME and macropinocytosis), one can rationally modulate complex siRNA-LBNPs towards the maximum siRNA delivery efficacy into cell cytosol. In order to optimize the siRNAs delivery into cell cytosol, the first approach is to increase the endo-lysosomal escape by modification of complex siRNA-LBNPs and the second one is to switch the existing pathways for complex siRNA-LBNPs towards non-lysosomal degradation.

Modification of complex siRNA-LBNPs can be obtained by the inclusion of polyethylene imine (PEI), 1,2-Dioleoylsn-glycero-3-phosphatidylethanolamine (DOPE), cell penetrating peptides (e.g., TAT and R8) during their formulation [11]. The interaction of PEI and siRNA forms a complex that can be further compounded with cationic lipids, resulting in the

ability of LBNPs to escape the endosomal compartments via proton sponge effect [22]. The nitrogen atoms within PEI can be protonated by utilizing endosomal protons that result in an increase in endosomal chloride anions, which diffuses into the endosomes with the protons and increase in osmotic pressure, thus inducing osmotic swelling. Thus, complex can escape from endosomes and release siRNA into cytosol. In addition, the addition of the pH sensitive fusogenic lipid (DOPE) within complex siRNA-LBNPs can release the siRNAs into cytosol [23]. At physiological pH, DOPE forms a stable lipid bilayer; however, at an acidic pH 5 to 6, it undergoes a transition from a bilayer to an inverted hexagonal structure, which fuses and destabilizes the endosomal membrane, releasing its contents to the cytosol. Moreover, the inclusion of CPPs into complex siRNA-LBNPs may be another possible way to enhance endosomal escape [11]. Most of CPPs (e.g., TAT, R8) contain a high density of basic amino acids (arginines and/or lysines), which are proposed to interact with the anionic surface of the plasma membrane and enhance internalization of the peptides. These peptides adopt an α-helical structure at endosomal pH leading to hydrophobic and hydrophilic faces that can interact with the endosomal membrane to cause disruption and pore formation.

Alteration of uptake mechanisms by modification of complex siRNA-LBNPs may be another way to decrease lysosomal degradation because some uptake pathways involve endo-lysosomes, while others are not associated with endo-lysosomes. Examples include CvME pathway that can bypass the endo-lysosomes. Therefore, stimulating this pathway to bypass endo-lysosomes is a new direction by which one can take the advantage of CvME to improve the efficiency of siRNAs. Several studies have shown that the siRNA-liposomes are taken up by cells through multiple endocytosis pathways, such as CME, CvME and macropinocytosis. We also found the similar results in the case of siRNA-liposomes. However, with the addition of a non-therapeutic amount of gold nanoparticle to siRNA-liposomes (auroliposomes), the uptake mechanism switched to mostly CvME, resulting in the significant improvement of silencing of a glycolytic switch in ovarian cancer [24]. By this way, it is possible to shift the uptake pathway to avoid lysosomal degradation. In addition, the size of caveolae is usually 50–100 nm in diameter; thus, there may be a possibility for smaller particles to be taken up by cells through CvME. In a previous study, authors showed that three particles with a size of 20, 40, and 100 nm were usually taken up by endothelial cells via CvME. The results demonstrated that both 20- and 40-nm nanoparticles were taken up by endothelial cells around 5–10 folds higher than that of the 100-nm particles, indicating that small particles can be taken up by CvME more effectively compared with large ones [25].

In addition, in CvME, the uptake of complex siRNA-LBNPs is mediated by the binding of specific ligands with the receptors. Several reports demonstrated that when the internalization of complex siRNA-LBNPs into cells is occurred via these αvβ3 and αvβ5 integrin [26], insulin [16], epidermal growth factor [17], and transforming growth factor beta [27] receptors, has been found to mediate this pathway. So, by this way the possibility of lysosomal degradation is abruptly diminished and improved the efficacy of siRNAs. Moreover, when cells face stresses, such as heat and hyperosmotic shock [28,29], it may be possible to stimulate caveolin internalization.

Conclusion

In a nutshell, various endocytosis pathways are associated with the uptake of complex siRNA-LBNPs and the subsequent delivery of siRNAs to cell cytosol, where siRNAs start further processing for their inhibitory function of target mRNA. Each pathway has its own intracellular events and that those events determine the fates of complex siRNA-LBNPs. Thus, the proper understanding of the uptake mechanisms is basically required for the rational design of complex siRNA-LBNPs. However, modulation of the uptake mechanisms towards CvME may be a fruitful direction for the successful delivery of siRNAs and their corresponding inhibitory activities against the target genes that are involved in the pathogenesis of diseases, including cancer, obesity, neurogenic disorders and pulmonary arterial hypertension. Since cellular uptake mechanisms predetermine the intracellular fates of complex siRNA-LBNPs, considering the advantageous pathways may offer the promising potential for improving the efficiency of siRNA delivery.

Competing Financial Interest

The authors declare that there is no completing financial interest.

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