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editorial

siRNA Delivery and Targeting

RNA interference (RNAi) is one of the most dramatic findings over the past decade, and the number of publications related to RNAi increased exponentially from 5 in 1998 to more than 2400 in 2008. Generally, RNAi can be achieved by three strategies: chemical synthesized small interfering RNA (siRNA), long double-stranded RNA (dsRNA), and DNA-based (plasmid or viral vector) short hairpin RNA (shRNA). Remarkable progress has been made in different aspects of the RNAi technology, and attempts are being made to use siRNA for therapeutic applications. In this issue, we will focus on the delivery and targeting of chemically synthesized siRNA. Currently, there are 12 ongoing clinical trials using siRNA for numerous diseases. Eight of these clinical trials employ naked siRNA for local treatment of ocular and respiratory diseases. Inefficient delivery to target cells and off-target effect are the two major barriers to turning siRNA into therapeutics. Off-target effect is not the focus of this issue, since significant progress has already been made in overcoming this problem.

This special issue contains several research and review articles, which highlight the recent advances in siRNA delivery and targeting. First, a thorough understanding of different biological barriers to siRNA therapeutics is essential for developing successful siRNA delivery system. Juliano and associates describe the major biological barriers between the initial administration of siRNAs and their final targets within cells, as well as possible strategies to overcome these barriers. The authors particularly point out the differences in barriers encountered by monomeric siRNA and siRNA encapsulated in nanocarriers. Nanocarriers efficiently deliver siRNA to tumor cells and other normal tissues with fenestrated endothelia, while molecular conjugates of siRNA with targeting ligands have advantages in targeting siRNA to specific cells or tissues after systemic administration.

Generally, an efficient siRNA delivery system includes a cationic group for ionic interaction with siRNA, poly(ethylene glycol) (PEG) for steric hindrance, an endosomolytic group for endosomal disruption, and a targeting ligand for site-specific delivery. The article by Huang and colleagues reviews the current delivery strategies for siRNA, especially the targeted, self-assembled nanoparticles which have shown a great potential for cancer therapy. Major components of these nanoparticles are cationic lipids and protamine which interact with the negatively charged siRNA. The surface of nanoparticles is modified with PEG, and the targeting ligand is attached to the distal end of PEG to achieve targeted delivery. In another paper, the same group describes a novel cationic lipid which can deliver siRNA and enhance its therapeutic effect in lung cancer cells. Lipid−polycation−DNA (LPD) nanoparticles were prepared with a non-glycerol based cationic lipid (DSGLA) which contains a lysine and guanidinium as the cationic headgroup. Compared to nanoparticles containing 1,2-*dioleoyl*-3-trimethylammonium-propane (DOTAP), nanoparticles prepared using DSGLA showed significant increase in tumor regression. Since all these conventional siRNA lipoplexes are formed electrostatically, it is possible that other charged molecules could disrupt the complexes before they reach target cells. Wagner and coworkers describe a strategy to conjugate siRNA directly to the poly(L-lysine) (PLL) backbone via a bioreducible disulfide bond which is stable in the presence of naturally occurring polyanions like heparin, but will be cleaved in the reducing intracellular environment. The other end of PLL is conjugated with an endosomolytic peptide which promotes the endosomal release of siRNA.

The most successful story for systemic delivery of siRNA is the self-assembling, cyclodextrin polymer-based nanoparticles developed by Mark Davis and his colleagues. Till now, this is the first targeted delivery system of siRNA which was approved for clinical trial in April 2008. This nanoparticle formulation contains a cyclodextrin-containing polycation, PEG, and human transferrin as a ligand for transferrin receptors on cancer cells. All these three components and siRNA self-assemble into nanoparticles which can be administered intravenously to patients. This article summarizes the discovery and translation of this approach all the way from concept to the clinic. It provides us new insights on how to design carriers based on the special characteristics of siRNA, and move the system into the clinic.

siRNA delivery to specific cell types is a key challenge for turning siRNA into therapeutics. A number of cell specific ligands including polysaccharide, antibody, antibody fragment, and peptide have been employed to achieve targeted delivery of siRNA. Lu and associates describe a siRNA nanocarrier modified with bombesin or RGD peptide via a PEG spacer. Systemic administration of this siRNA formulation showed a significant tumor growth inhibition compared to nontargeted siRNA nanocarrier or naked siRNAs. Although most targeted siRNA delivery systems are formed by surface modification of the siRNA complex with targeting ligands, siRNA can also be directly attached to a targeting ligand. Pardridge and colleagues attached siRNA to a monoclonal antibody against the human insulin receptor via a stable avidin−biotin linkage. This siRNA conjugate showed comparable silencing effect compared to siRNA delivered with cationic polymers.

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Not only the cellular uptake but also the location of siRNA within the cells is critical for its silencing effect. Only the intact siRNAs in the cytoplasm are able to silence the target mRNA. The paper by Miller and associates describes a hepatotropic lipid-based system for siRNA delivery to the liver. PEG was modified to the lipoplex surface with a pH-sensitive oxime linkage, which could facilitate acidic pH-triggered release of siRNA from endosomes. Knockdown of hepatitis B virus (HBV) replication was observed in cell culture and mice after hydrodynamic injection. Bull and co-workers describe another delivery system which can enhance siRNA localization to the cytoplasm. This system contains a bioreducible cationic polymer which can be cleaved by the reductive environment of the cytoplasm, and releases the encapsulated siRNA. They showed enhanced silencing effect on target gene in various cancer cell lines using this system compared to the control system which shows the same cellular uptake, but less distribution in the cytoplasm. This is in accordance with our finding that the level of gene silencing effect does not increase proportionally with the siRNA dose. The gene silencing ability of siRNAs had no linear relationship with their dose. To facilitate the rational design of siRNA delivery, Chen and his colleagues describe a new mechanism for cationic lipid-mediated siRNA delivery. They found that inhibition of endocytosis or macropinocytosis failed to block the silencing effect of siRNA although ~95% of siRNA enter the cells through endocytosis. On the contrary, depletion of cholesterol from the cell membrane significantly abolishes the silencing effect of siRNA, but has little effect on the cellular uptake. This finding demonstrates that the fusion between siRNA lipoplexes and the cell membrane may account for the functional siRNA delivery. All these results suggest that understanding of cellular and subcellular uptake mechanisms of siRNA will help us develop efficient siRNA therapeutics.

Light-controllable strategy is another interesting method to achieve spatial and temporal targeting of siRNA. An article by Monroe and co-workers discusses numerous cage compounds with different photochemical properties and strategies for their applications. The common way to achieve photocontrol is to add a photolabile cage compound to siRNA so that the silencing effect is blocked until triggered with light exposure. Both selection of the cage compound and conjugation site within siRNA are critical for its successful application.

Finally, we would like to express our sincere appreciation to all contributors of this special issue of *Molecular Pharmaceutics*. Through this issue, case studies of various delivery systems and comprehensive reviews offer insight into this rapidly developing area. The future holds great promise for siRNA therapeutics although there is still a long way to go before it become a mature technology to treat different diseases. It is widely accepted that the collaboration among scientists with different backgrounds is very critical to overcome those problems associated with the development of siRNA therapeutics. We hope that the delivery strategies described in this issue will enhance our understanding and may spark some new ideas in developing siRNA therapeutics.

Kun Cheng
Guest Editor

Division of Pharmaceutical Sciences, University of Missouri—Kansas City, 5005 Rockhill Road, Kansas City, Missouri 64110
E-mail: chengkun@umkc.edu

Ram I. Mahato
Guest Editor

Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 19 South Manassas, RM 224, Memphis, Tennessee 38103
E-mail: rmahato@utmem.edu

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