31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Traceable siRNA delivery with quantum dots

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Abstract— **A new generation of drug carrier that allows efficient delivery and real-time imaging of siRNA in live cells has been developed by combining nanoparticles and amphiphilic polymers. Compared with the classic siRNA carriers such as Lipofectamin and polyetheleneimine, this new class of nanocarrier works in both serum-free and complete cell culture media, which is advantageous over Lipofectamine. It also outperforms polyethyleneimine in gene silencing under both conditions with significantly reduced toxicity. Furthermore, the core nanoparticles provide a mechanism for real-time imaging of siRNA delivery. This new multifunctional, compact, and traceable nanocarrier is expected to yield important information on rational design of siRNA carriers and to have widespread applications of siRNA delivery and screening in vitro and in vivo.**

Keywords—**nanoparticles, siRNA, delivery, imaging, targeting**

I. Introduction

RNA interference (RNAi) is emerging as one of the most powerful technologies for sequence-specific suppression of genes and has potential applications ranging from functional gene analysis to therapeutics. Due to the relatively low immunogenic and oncologic effects, the development of non-viral delivery methods in vitro and in organisms is of considerable current interest. In recent years, a number of strategies have been developed based on liposomes, gold and silica nanoparticles (NPs), cationic and biodegradable polymers, and peptides. The delivery efficiency, however, remains low, especially under in vivo conditions. Another limitation shared by all the existing delivery technologies is the lack of an intrinsic signal for long term and real-time imaging of siRNA transport and release. Such imaging could provide important information on rational design of siRNA carriers. Currently, organic fluorophores are used to label siRNA or the delivery vehicles. But the photobleaching problem associated with essentially all organic dyes prevents long-term tracking of siRNA-carrier complexes.

In this context, the use of semiconductor quantum dots (QDs) to study siRNA delivery in cells and small animals should be an excellent choice because of QDs' intrinsic fluorescence and their unique optical properties (e.g., tunable emission, photostability and brightness). Here, we report the development of multifunctional nanoparticles with integrated functionalities of cell binding and internalization, endosome escape, siRNA protection against enzyme activities, siRNA unpackaging (siRNA-carrier dissociation), and siRNA tracking. Packaging these functionalities into single nanoparticles also represents a significant technological challenge.

II. Methodology

We have developed a new technology by combining QDs with amphipol to solve the aforementioned problems. Amphipols are linear polymers with alternating hydrophilic and hydrophobic side chains. They are widely used for solubilizing integral membrane proteins and delivering them into cell lipid bilayers. Unlike detergent-based micelles, amphipols belt around the transmembrane domain of membrane proteins and do not disrupt the integrity of cell membrane during delivery. When amphipols are mixed with nanoparticles coated with hydrophobic surface ligands, these two types of nanomaterials form stable complexes that are not only capable of carrying siRNA molecules into cytoplasm but also protecting them from enzymatic degradation. In addition, the QDs should also provide a bright and stable fluorescent signal for intracellular siRNA imaging, since great success has been achieved in the past 5 years in using QDs for cellular staining and imaging. The QD-amphipol technology reported here will open new opportunities for traceable intracellular delivery of siRNA without the need of additional compounds

III. Results

We selected poly(maleic anhydride-alt-1-decene) modified with dimethylamino propylamine (PMAL) because of its multiple useful properties. First, the hydrocarbons in PMAL bind to the hydrocarbons on the surface of QDs via multivalent hydrophobic interactions, leading to the formation of stable and water-soluble organic-inorganic hybrid structures. Second, at neutral pH, the overall surface charge of the hybrid structure is highly positive, which allows immobilization of negatively charged biomolecules (e.g., siRNAs) and interaction with negatively charged cell surface. Previously, we and others have prepared amphiphilic copolymers for QD solublization and bioconjugation for cell labeling. But all those polymers employ a dense layer of carboxylic acids, which prevents interaction with siRNA molecules. Third, the clustered tertiary amines grafted on the PMAL backbone have strong proton absorbing capability inside acidic cellular compartments such as endosomes, leading to osmotic swelling and endosome rupture. Fourth, the co-existence of tertiary amine and carboxylic acid groups weakens the interaction between siRNA and nanoparticles, which is

expected to facilitate siRNA release inside cells. Indeed, it has been found that when polyethylenimine (PEI) are chemically modified to reduce electrostatic binding, the gene delivery activity is increased by 20-60 fold. Furthermore, the zwitterionic surface of QD-PMAL could also become an important feature for in vivo applications, because zwitterionic charge reduces serum protein adsorption onto NP surface, which not only slows NP uptake by the reticuloendothelial systems (RES), but also helps NP renal clearance when the particles are made smaller than 5.5 nm.

To evaluate the RNAi efficiency using QD-PMAL delivery vehicle, a model gene silencing experiment was designed using human breast adenocarcinoma cell line (SK-BR-3) and siRNA targeting Her-2/neu. Her-2/neu, a cell surface receptor tyrosine kinase, is over-expressed in approximately 30% of breast tumors, and is an excellent model system because it is involved in signal transduction pathways leading to cell growth and differentiation. Her-2/neu expression was suppressed to 36±2% using QD-PMAL in serum-free media. In comparison, when the two common transfection reagents (Lipofectamine and PEI) were used, the target gene expression was reduced to 29±5% and 58±13%, respectively. When used in complete cell culture media (contains serum), QD-PMAL reduces Her-2 expression to 35±4%, similar to the values achieved with serum-free media. Lipofectamine and PEI reduce Her-2 expression to 48±7% and 62±5%. These results demonstrate that QD-PMAL is efficient in siRNA intracellular delivery for both serum-free and complete media. In contrast, Lipofectamine only works well in serum-free media, and the QD-PMAL also outperforms PEI under both conditions.

The unique optical properties of QDs and exquisite design of imaging assays (such as FRET) allow real-time imaging of siRNA delivery in live cells. Further development of this new multifunctional, compact, and traceable nanocarrier (such as incorporating a targeting probe) will enable new developments in siRNA discovery and delivery, functional genomics, gene therapy, as well as biophysical studies.

IV. Discussion

Although QD-based optical imaging provides high sensitivity and resolution in cells and small animals, it's not highly suitable for deep tissue imaging because of limited light penetration depth. In addition, high quality QDs often contains toxic elements such as cadmium. Although the amphipol coating can keep the QDs stable and intact for at least several months, the long term toxicity (years) is largely unknown. This problem could be solved by using nanoparticles of similar size and surface properties, and of no or low toxicity (e.g., iron oxide magnetic nanoparticles).

REFERENCE

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Acknowledgment

This work was supported in part by NIH, NSF, and the Department of Bioengineering at the University of Washington. X.G. thanks the NSF for a Faculty Early Career Development award (CAREER).