Amphiphilic DNA and its application in biomedicine

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Summary

Breakthroughs in lipid DNA, DNA conjugated with hydrophobic moieties, have established it as a versatile functional material in many different fields. The interplay between unique structural and chemical properties of nucleic acids and hydrophobicity enables lipid DNA with novel functionalities and broadens its application in the field of nanotechnology, diagnostics and biomedicine. In **Chapter 1**, current state of the DNA amphiphiles synthesis and their assembly into nanostructures were summarized. Next, an overview on the interaction of these DNA amphiphiles with membranes was provided, detailing on the driving forces and the stability of this interaction. Moreover, the interaction with cell surfaces in respect to therapeutics, biological sensing, and cell membrane engineering was highlighted. Finally, the challenges and outlook on this promising class of DNA hybrid materials were addressed.

In **Chapter 2**, lipid DNA, dodec-1-yne-modified deoxyuridine nucleotides incorporated into common DNA sequences, was synthesized and its interaction with both liposomal and cellular membrane was presented. Förster resonance energy transfer method proved that this lipid DNA was able to anchor in liposomal membranes, either in unsaturated (DOPC:DOPE:cholesterol = 2:1:1) or saturated liposome (DPPC:cholesterol = 2:1) formulations. This method also indicated that liposomal membrane anchored lipid DNA is accessible to DNA hybridization. Even when 5% PEG was incorporated into liposome composition, lipid DNA was still successfully anchored and hybridized with its complementary strand with high efficiency. After confirming the capability of lipid DNA anchoring in liposomal membranes, we further challenged the system to anchor to cellular membranes. After a short incubation with HeLa cells, lipid DNA quickly pierced into cellular membranes and the membrane anchored DNA again was accessible to hybridization with its complementary strands, as indicated by flow cytometry measurements. Confocal fluorescence microscopy images showed that comple-

mentary strands of lipid DNA were homogenously distributed on the cellular membrane. Again this proved that the lipid DNA was anchored in the membrane. We also measured the anchoring stability and found that lipid DNA stably incorporated into the membrane for several hours. When increasing the hydrophobicity of lipid DNA by adding more modified nucleotides, its membrane anchoring stability was increased. These findings suggested possible applications of lipid DNA in biomedicine, thus we were prompted to evaluate its cellular biocompatibility. We found that the toxicity of lipid DNA was concentration dependent and at 10 uM concentration, its toxicity was acceptable.

In **Chapter 3**, a fast, efficient and targeted liposome delivery system controlled by DNA hybridization was presented. We anchored cells and liposomes with lipid DNA respectively, and then incubated them together for only 15 mins. When lipid DNA on liposomal membrane was complementary to that on cellular membrane, cellular internalization of liposomes was increased 18 times as compared with the non-hybridized control, as quantified by flow cytometry. The following dynamic study indicated that liposomes entered cells as quickly as in 5 min while for the non-hybridized control, no obvious uptake could be detected even after 30 min. Due to the high specificity of DNA hybridization, we found liposome could be only delivered to cells whose membrane was pre-labeled with complementary lipid DNA strands. When two types of liposomes were mixed with two populations of cells, precise recognition by DNA hybridization directed liposomes to the targeted cells. The mechanism of liposome delivery was also investigated. Liposome uptake was significantly reduced when internalization experiments were conducted at low temperature. This indicated an energy-dependent internalization process. Further treating cells with different endocytosis inhibitors showed that caveolae-mediated endocytosis was the dominant liposome internalization pathway and scavenger receptors also assisted the cellular uptake. Finally, cellular track of liposome and its cargo revealed liposome fate after internalization as follows: liposomes first accumulate on cellular membranes by DNA hybridization. With the help of caveolae and scavenger receptors, surface tethered liposome enter cellular endosome. Once reaching the lysosome, liposomes are degraded, and its cargo is released.

After confirming that DNA hybridization could promote liposome attachment to the cellular membrane and enhance cellular internalization, we further explored to generalize this method from liposomes to other types of nanoparticles. Therefore, in **Chapter 4**, a simple DNA nanostructure, i.e. a DNA tetrahedron, was tailed with free overhangs. When incubated with HeLa cells pre-anchored with lipid DNA, a significant amount of cellular DNA tetrahedron was observed while for non-hybridized controls, no obvious internalization was detected. Flow cytometry results conformed this enhanced uptake, which was as high as 100 times. Moreover, it was found that the internalization of DNA tetrahedron was concentration dependent. A time series of incubation of DNA tetrahedron with growth medium indicated its structural stability, meaning that it is stable up to 5 hours. We also synthesized 13 nm AuNPs conjugated with oligonucleotides on the surface. Similar as for the DNA tetrahedron, AuNPs internalization was increased with the help of surface DNA hybridization, as confirmed by dark-field microscopy. To further confirm this DNA hybridization method could be applied as a general way to increase nanoparticle internalization, another type of nanoparticle, i.e. polystyrene nanoparticles, was also modified with free oligonucleotides and its cellular internalization was studied. We found that DNA hybridization could promote cellular uptake of this type of nanoparticle as well.

Apart from inserting its hydrophobic tail into membranes, the DNA segment of lipid DNA can be designed to be bio-functional. In **Chapter 5**, lipid DNA with CpG sequence was synthesized. It can either self-assembly into nano-sized micelle to form an immunostimulatory micelle or insert into liposomal membranes to represent an immunostimulatory liposome. To compare their immunostimulatory effects with conventional nanoparticles, 13 nm AuNPs with CpG sequence conjugated was also synthesized. These three types of nanoparticles were injected into mice and their dendritic cells activation ability was evaluated. It was found that immunostimulatory micelles performed best and could effectively promote immune activation, as indicated by elevated expression of co-stimulatory molecules and secretion of pro-inflammatory cytokines.