Combinatorial Cationic Lipid-like Nanoparticles for Efficient Intracellular Cytotoxic Protein Delivery

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Introduction: Although protein-based drugs have shown success, they have been limited mostly to cytokines, growth factors, enzymes and monoclonal antibodies, all of which function primarily extracellularly. There are a number of diseases, including genetic diseases and cancers, that have the potential to be treated through proteins with an intracellular target. However, proteins alone are not usually able to cross the cell membrane in order to reach their intracellular targets. Hence it is desirable to develop efficient and effective tools as well as strategies that will enable us to deliver therapeutic proteins in their active forms to tumor cells or tissues. Here we present a combinatorial approach for the creation of cationic lipid-like nanoparticles (termed "lipidoids") to facilitate intracellular cytotoxic protein delivery for the inhibition of tumor cell proliferation.

Methods: *Synthesis of lipidoids.* In a 5 mL Telfon-linked glass screw-top vial, 1, 2-epoxyoctadecane and different amines were mixed at molar ratios of 2.4: 1(amine:epoxy), followed by a two-day reaction at 90 ^{o}C without solvent. After cooling, the lipidoid mixtures were purified through flash chromatography on silica gel and characterized by ^{1}H NMR.

Cell culture. All cells lines in this investigation were purchased from ATCC (Manassas, VA) and cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37 ^{o}C in the presence of 5% CO_2. For protein transfection experiments, cells were seeded in 96-well plates at a density of 10,000 cells per well a day prior to transfection.

Modification of RNase A and saporin: The proteins, (RNase A and saporin) were modified with *cis*-aconitic anhydride in 0.1 M $NaHCO_3$ buffer solution (pH = 9.5). The crude products were purified with Amicon Ultra for three times.

In Vitro Protein Transfection. The lipidoid/protein complexes were prepared simply by adding lipidoid to the phosphate buffer solutions of proteins at varied mass ratios. After the addition of the lipidoid/protein complexes to the B16F10, cells were incubated at 37 ^{o}C for an additional 48 h. The cell viability was determined by MTT assay after 48 h of incubation. All transfection experiments were performed in quadruplicate.

Results and Discussion: In order to obtain the protein delivery efficiency of different lipidoids, we measured the viability of B16F10 cells exposed to various lipidoid/protein complexes and compared them to blank controls. As shown in Figure 1, the viabilities of B16F10 cells treated with lipidoids are comparable to the control, indicating the low cytotoxicity and biocompatibility of the lipidoids. Similarly, when the cells were incubated with the four proteins in the absence of any lipidoids, no significant toxicity was observed due to the lack of efficient cellular uptake pathways of proteins. However, lipidoid-protein complex treated cells showed distinct viabilities and depend on the lipidoid and protein type. No appreciable viability decrease was observed for the cells exposed to lipidoid/RNase A mixtures, indicating a low RNase A delivery efficiency by all lipidoids in the library. In contrast, RNase A-Aco, saporin, and saporin-Aco can be delivered and induce significant cytotoxicity with seven lipidoids in the library (EC16-1, EC16-3, EC16-4, EC16-5, EC16-6, EC16-12, and EC16-14). The lipidoids are named EC16 followed by the amine number in the library, where EC16 indicates 1, 2-epoxyoctadecane. A notable example of delivery efficiency is EC16-1, which can facilitate delivery of RNase A-Aco, saporin, and saporin-Aco reducing cell viability down to 30%. It is also noteworthy that EC16-1 delivered saporin and saporin-Aco at almost the same efficiency, indicating the cationic lipidoid can interact with both positively and negatively charged saporin.

The optimal protein transfection conditions, including dose, ratio, cell line, were studied. The biodistribution of protein-lipidoid nanoparticle was also evaluated in tumor xenografted nude mice model.

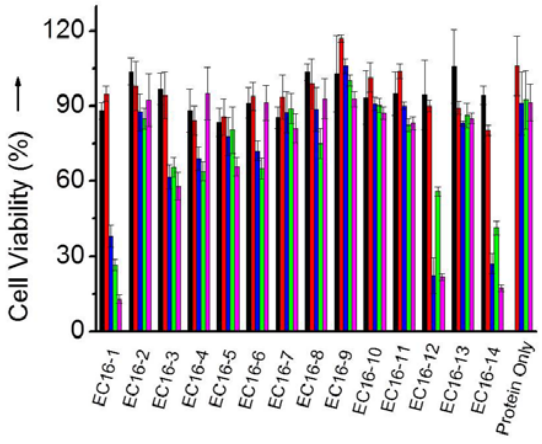

Figure 1. Evaluation of lipidoid-facilitated protein delivery on B16F10 cell line via cytotoxicity assay. Black: Lipidoid controls; Red: RNase A; Blue: RNase A-Aco; Green: saporin; Magenta: Saporin-Aco. The cytotoxicity was determined by MTT assay.

Conclusion: we report the use of combinatorial-designed cationic lipid-like nanoparticles for the facilitation of intracellular protein delivery and its potential use for cancer therapies. The capabilities of these lipidoids to facilitate protein delivery are demonstrated by delivery of two cytotoxic proteins, RNase A and saporin, into various cancerous cell lines. Such combinatorially-designed lipidoids could be extended into in vivo applications of protein delivery for cancer therapy.