The Chinese University of Hong Kong
Department of Biomedical Engineering

Graduate Seminar – PhD Oral Defence

Student	***:***	Miss LI Huize
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Date	***:***	20 October, 2020 (Tuesday)
Time	***:***	10:00 am
Zoom Link	***:***	https://cuhk.zoom.us/j/98653261952?pwd=azBqZkNPRCtXeTd3L1FvUUdWTkZSUT09
Meeting ID	***:***	986 5326 1952
Password	***:***	288800

Title: Intranuclear Delivery of Oligonucleotide-based Nanostructures via Gentle Compression and Attachment of Polythymidine

Delivery of nucleic acids to the cell nucleus not only supports investigations into intracellular biological mechanisms at the genetic level, but also considered to be a promising strategy of disease treatment by regulating the expression of target genes. Intranuclear delivery of nucleic acids remains a significant challenge due to the need to penetrate both the cell membrane and nuclear membrane. Nonviral delivery of nucleic acids to the cell nucleus typically relies on chemical methods that do not guarantee specific delivery (e.g., transfection agent) or physical methods that may require extensive fabrication (e.g., microfluidics) or an elevated pressure (e.g., 10^5 Pa for microneedles).

In our initial studies, we demonstrate the intranuclear delivery of one-dimensional (1D) nucleic acids. We develop a "compression-cum-polythymidine" ("compressioncum-poly(T)") method of delivering oligonucleotides to the nucleus with high specificity (relative to the cytosol) by synergistically combining chemical and physical approaches. Particularly, we demonstrate that oligonucleotides appended with a polythymidine [poly(T)] segment (chemical) profusely accumulate inside the nucleus when the cells are under gentle compression imposed by the weight of a single glass coverslip (physical; ~2.2 Pa). Our "compression-cumpoly(T)" method is simple, can be generalizable to three different mammalian cell types that are difficult to be transfected by non-viral methods (bEnd.3 endothelial cells, Kera-308 keratinocytes, and RAW264.7 macrophages). By tuning three experimental parameters (applied pressure, compression time, and oligonucleotide concentration), we achieve intranuclear delivery of poly(T) oligonucleotides of 30 bases (denoted T30) without inducing severe cytotoxicity or oxidative stress to the three cell types after 48 h of recovery. In bEnd.3 cells, by employing a genetic knockdown approach, we prove that importin β and nucleoporin 62 mediate the transport of T30 to the cell nucleus. Our method significantly enhances the intranuclear delivery of antisense oligonucleotides to bEnd.3 cells and the inhibition of two target genes, including an exogenous reporter gene encoding the enhanced green fluorescent protein (EGFP) and an intranuclear lncRNA oncogene [metastasis associated lung adenocarcinoma transcript 1 (MALAT1)], when compared to delivery without gentle compression or poly(T) attachment.

Next, we demonstrate the application of our "compression-cum-poly(T)" method to achieve intranuclear delivery of "spherical nucleic acids (SNAs)", three-dimensional (3D) nucleic acid nanostructures. We prove the localization of ~13 nm poly (T)-coated SNAs in the nucleus, typically with an intranuclear fraction of up to ~50% depending on the cell type by subjecting the cells to gentle compression. We realize the intranuclear delivery of ~13 nm poly(T)-coated SNAs in bEnd.3, Kera-308, RAW264.7, and HeLa cells, and prove that importin β mediate the transport of poly(T)-containing SNAs to the nucleus of cells. To study mechanosensation during compression, we detected the expression of GTP-bound RhoA (active form) increased in response to compressive stress in cells. By pharmacological blocking, we have further shown that the nucleus entry of SNAs involves RhoA-signaling. By RNA sequencing, we proved that the nucleus import of cargoes by our "compression-cum-poly(T)" method is mediated by the importin, with involvement of "Regulation of actin cytoskeleton" pathway to speed up the process of intranuclear delivery. We finally apply our method to enhance the intranuclear delivery of SNAs to bEnd.3 cells and the inhibition of EGFP.

Our "compression-cum-poly(T)" method will open the avenue for the intranuclear delivery of antisense oligonucleotides and potentially other types of nucleic acids (e.g., siRNAs) that target other genes to a wide variety of cell types (e.g., different morphologies). Our data reveal the interplay between intracellular trafficking and cellular biomechanics.

***** ALL ARE WELCOME *****

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