Illuminating the Mechanistic Pathways of Polymer-Mediated Nucleic Acid Delivery

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The wealth of information being obtained from genomic, proteomic, and glycomic research is allowing researchers to unravel the intricate genetic and epigenetic mechanisms associated with human health and disease. The intracellular delivery of nucleic acids to study these processes offers unprecedented promise for revolutionizing biomedical research and novel drug development. However, the nucleic acid delivery vehicle plays a central yet elusive role in dictating the efficacy, safety, mechanisms, and kinetics of gene regulation in a spatial and temporal manner; thus having a far-reaching impact in health-related research. We have developed several novel carbohydrate-based cationic polymers that have shown outstanding affinity to bind and encapsulate oligonucleotides and plasmid DNA into nanocomplexes (polyplexes) that facilitate highly efficient intracellular delivery while minimizing toxicity. We aim to further understand our wide-range of polymer delivery vehicles for their mechanistic pathways and kinetics of nucleic acid encapsulation and intracellular transport from the cell surface to their final intracellular destination. Our New Innovator Award Program has been driven by three specific goals: 1) to unravel the molecular-level interactions between structurally-diverse yet analogous polymeric delivery vehicles and differing nucleic acid types and to correlate these interactions with the biological stability and mechanisms of the subsequent polyplexes, 2) to understand the interactions of these various polyplex types with cell surface glycosaminoglycans and compare polyplex structure to receptor selectivity and mechanisms of cellular uptake, and 3) to decipher the intracellular trafficking pathways in a spatial and temporal manner from uptake to the final destination for each polyplex form. Glycopolycation vehicles have been synthesized that vary in carbohydrate type and cationic functionality. We show that the polymer structure significantly impacts the ability of the vehicle to bind and compact various nucleic acids (siRNA and pDNA) into polyplexes and polyplex structure dictates nanocomplex colloidal stability. The role of polymeric chemistry on intracellular trafficking and in vivo delivery has been studied via several methods. Polyplexes were found to associate with heparan sulfate on the cell surface and are internalized by endocytic mechanisms involving both caveloae and clathrin. Immunofluorescence images indicate that some polymer vehicles traffic nucleic acids to the Golgi and endoplasmic reticulum (ER), and colocalization experiments indicated retrograde transport of polyplexes *via* COP I vesicles from the Golgi to the ER. Multiple high resolution tomographic images of whole cells were captured via confocal microscopy at various timepoints. The images were reconstructed to visualize and quantify trafficking kinetics *in situ* in a spatio-temporal manner.