MOLECULAR DESIGN AND NANOPARTICLE-MEDIATED INTRACELLULAR DELIVERY OF FUNCTIONAL PROTEINS TO TARGET CELLULAR PATHWAYS

by

Dhiral Ashwin Shah

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Examining Committee:

Prof. Ravi S. Kane, Thesis Advisor

Prof. Jonathan S. Dordick, Thesis Advisor

Prof. Steven Cramer, Member

Prof. George Plopper, Member

Rensselaer Polytechnic Institute
Troy, New York

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ABSTRACT

Intracellular delivery of specific proteins and peptides represents a novel method to influence stem cells for gain-of-function and loss-of-function. Signaling control is vital in stem cells, wherein intricate control of and interplay among critical pathways directs the fate of these cells into either self-renewal or differentiation. The most common route to manipulate cellular function involves the introduction of genetic material such as full-length genes and shRNA into the cell to generate (or prevent formation of) the target protein, and thereby ultimately alter cell function. However, viral-mediated gene delivery may result in relatively slow expression of proteins and prevalence of oncogene insertion into the cell, which can alter cell function in an unpredictable fashion, and non-viral delivery may lead to low efficiency of genetic delivery. For example, the latter case plagues the generation of induced pluripotent stem cells (iPSCs) and hinders their use for *in vivo* applications. Alternatively, introducing proteins into cells that specifically recognize and influence target proteins, can result in immediate deactivation or activation of key signaling pathways within the cell.

In this work, we demonstrate the cellular delivery of functional proteins attached to hydrophobically modified silica (SiNP) nanoparticles to manipulate specifically targeted cell signaling proteins. In the Wnt signaling pathway, we have targeted the phosphorylation activity of glycogen synthase kinase-3β (GSK-3β) by designing a chimeric protein and delivering it in neural stem cells. Confocal imaging indicates that the SiNP-chimeric protein conjugates were efficiently delivered to the cytosol of human embryonic kidney cells and rat neural stem cells, presumably *via* endocytosis. This uptake impacted the Wnt signaling cascade, indicated by the elevation of β-catenin levels, and increased transcription of Wnt target genes, such as *c-MYC*. The results presented here suggest that functional proteins can be delivered intracellularly *in vitro* using nanoparticles and used to target key signaling proteins and regulate cell signaling pathways.

The same concept of naturally occurring protein-protein interactions can also be implemented to selectively bring intracellular protein targets in close proximity to proteasomal degradation machinery in cells and effect their depletion from the cellular

compartments. This approach will be able to not only target entire pool of proteins to ubiquitination-mediated degradation, but also to specific sub-pools of posttranslationally modified proteins in the cell, provided peptides having distinct binding affinities are identified for posttranslational modifications. This system can then be tested for intracellular protein delivery using nanoparticle carriers to identify roles of different posttranslational modifications on the protein's activity. In future work, we propose to develop a cellular detection system, based on GFP complementation, which can be used to evaluate the efficiency of different protein delivery carriers to internalize proteins into the cell cytosol. We envision the application of nanoscale materials as intracellular protein delivery vehicles to target diverse cell signaling pathways at the posttranslational level, and subsequent metabolic manipulation, which may have interesting therapeutic properties and can potentially target stem cell fate.