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Editorial

# Drug nanorods are potential new nanocarriers for intracellular protein delivery

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## Abstract

Protein therapeutics are increasingly important for the treatment of many diseases; however, intracellular protein delivery remains a considerable challenge. To address this challenge, drug nanorods have emerged as new nanocarriers for enhanced intracellular protein delivery via bypassing endo-lysosomes, which was called a "drug-delivering-drug platform".

Key words: protein therapeutics; protein delivery; nanocarrier; nanomedicine

Protein therapeutics become popular in the treatment of human diseases including diabetes, cancer, infectious and inflammatory diseases, due to their high activity and high specificity as compared to small molecule drugs. Nevertheless, protein therapeutics suffer from poor stability, short circulatory half-life, poor-membrane permeability, and immunogenicity [1,2]. To address these problems, many strategies have been developed for protein delivery, including polymer conjugation, long-circulating protein fusion, and nanocarriers such as nanogels, nanocapsules, virus-like particles, and cationic lipid nanoparticles. Indeed, dozens of PEGylated protein therapeutics, human serum albumin (HSA) fused protein therapeutics, and fragment crystallizable (Fc) fused protein therapeutics have been approved by the U.S. Food and Drug Administration (FDA) [3,4]. However, current FDA-approved protein therapeutics aim at extracellular targets, not at intracellular ones. This is because it is tremendously difficult for therapeutic proteins to spontaneously enter cells and efficiently escape from the endo-lysosomal system into the cytosol to play roles [5-7]. Although much effort has been directed to overcome these problems in intracellular protein delivery [8-10], it remains a significant challenge to efficiently deliver therapeutic proteins into the cytosol of cells.

Recently, Xiaofei Xin *et al*. reported a drug-delivering-drug platform (HA-PNPplex) for intracellular delivery of protein and combined cancer treatment [11]. In their work, rod-like pure drug nanoparticles (PNPs) of paclitaxel (PTX) stabilized with positively charged polymer polyethylenimine (PEI) were used as nanocarriers of proteins *via* electrostatic interactions between the positively-charged PNPs and the proteins to form PNPs/protein complexes (PNPplex). Subsequently, hyaluronic acid (HA) was coated onto these PNPplex to yield HA-PNPplex *via* electrostatic interactions between the PNPplex and HA. To prove the concept, caspase 3, the dominant mediator of apoptosis in mammalian cells [12,13], was chosen for the demonstration of therapeutic efficacy in a caspase-3-deficient MCF-7 tumor model. They found that HA-PNPplex delivered caspase 3 into cells through a non-lysosomal pathway, the caveolae-mediated internalization, which was determined by the rod-like shape, not by the surface modification. Consequently, HA-PNPplex raised caspase 3 level up to 6.5-fold in MCF cells. In a MCF-7 breast cancer mouse model, HA-PNPplex showed synergistic effect between the protein and PTX. The authors claimed that the platform provides a

completely new strategy for protein therapies and delivery for other biomacromolecules, even for cancer immunotherapy.

These data appear to be interesting for protein delivery; however, several issues need to be addressed in the future. First, PEI is believed to be toxic [14], although the authors claimed that HA-PNPplex had high biocompatibility and tolerability. We would suggest that biodegradable and biocompatible positively-charged polymers like polylysine should be selected instead of PEI. Second, many proteins are not negatively-charged or not negative enough to form stable complexes with positively-charged polymers through electrostatic interactions as mentioned in this work. Therefore, the authors should clarify this limitation in their paper. Third, it is not clear that how much HA could be adsorbed onto the surface of PNPplex and how stable HA-PNPplex was. Therefore, there are several subtle or uncertain factors to be considered in the preparation of HA-PNPplex, which may complicate the quality control of HA-PNPplex in manufacturing. To our knowledge, more and more nanomedicines were composed of more than two components like this work [15,16]. The structures of the nanomedicines were usually not clear because it is very difficult to well characterize these nanostructures, especially *in vivo*. More attention should be paid to this problem from the viewpoint of translational medicine. Fourth, the tumor penetration of HA-PNPplex was not well studied in this work. A reasonable method to investigate tumor penetration is to measure the distance of protein migration from the vessel [17,18]. Fifth, the authors claimed that PNPs would be useful to carry antigen proteins for cancer immunotherapy. One question is whether it is necessary or reasonable to use PNPs as nanocarriers for antigen delivery. The other question is whether PNPs are toxic to immune cells.

In our opinion, this work provides new nanocarriers of PNPs for efficient intracellular delivery of some negatively-charged proteins *via* caveolae-mediated endocytosis and shows a synergy of PTX therapy and caspase 3 therapy. After solving the above problems, the nanocarriers of PNPs would be useful for combined cancer therapy. To date, many nanocarriers have been developed for intracellular protein delivery, but most of them are complicated in structures and functions along with insufficient characterization, which may result in poor reproducibility and further hinder their clinical translation. We believe that less is more, which is especially important for drug development.

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## Competing Interests

The authors have declared that no competing interest exists.

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