Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Recent developments in intracellular protein delivery

Yumiao Zhang[1], Joachim Justad Røise[2], Kunwoo Lee[3], Jie Li[1] and Niren Murthy[1]

Protein therapeutics based on transcription factors, gene editing enzymes, signaling proteins and protein antigens, have the potential to provide cures for a wide number of untreatable diseases, but cannot be developed into therapeutics due to challenges in delivering them into the cytoplasm. There is therefore great interest in developing strategies that can enable proteins to enter the cytoplasm of cells. In this review article we will discuss recent progress in intracellular protein therapeutics, which are focused on the following four classes of therapeutics, Firstly, vaccine development, secondly, transcription factor therapies, thirdly, gene editing and finally, cancer therapeutics. These exciting new advances raise the prospect of developing cures for several un-treatable diseases.

Addresses
[1] Department of Bioengineering, University of California, Berkeley, Berkeley, CA 94720, USA
[2] Department of Chemistry, University of California, Berkeley, Berkeley, CA 94720, USA
[3] GenEdit, Berkeley, CA 94720, USA

Corresponding author: Murthy, Niren (nmurthy@berkeley.edu)

Current Opinion in Biotechnology 2018, **52**:25–31

This review comes from a themed issue on **Tissue, cell and pathway engineering**

Edited by **David Schaffer** and **Stanislav Y Shvartsman**

https://doi.org/10.1016/j.copbio.2018.02.009

Introduction

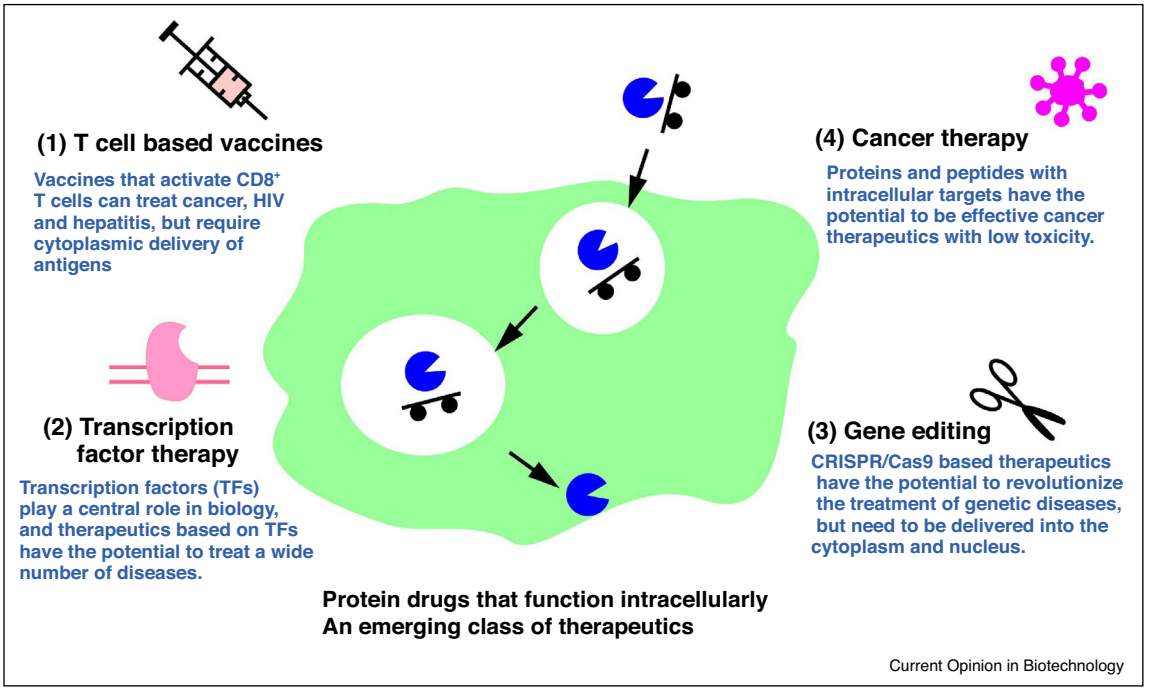
Protein based therapeutics, such as antibodies, receptor decoys and cell surface ligands have transformed the field of drug development and have the potential to impact all areas of medicine. However, despite their success, the full potential of protein based therapeutics has still not been realized because it has been impossible to develop protein therapeutics that target intracellular biomolecules. For example, therapeutics based on transcription factors, gene editing enzymes, metabolic enzymes and protein antigens, have the potential to provide cures for a wide number of untreatable diseases, but cannot be developed into therapeutics due to challenges in delivering them into the cytoplasm.

There is therefore great interest in developing strategies that can enable proteins to enter the cytoplasm of cells. However, despite 40 years of research the development of therapeutics that work intracellularly remains a major challenge. Delivering proteins intracellularly is challenging because it requires developing multifunctional delivery vehicles that can perform the functions of triggering endocytosis and endosomal disruption and this frequently requires assembling macromolecular complexes. This is inherently challenging with protein therapeutics because of their large size, their sensitivity to chemical modifications and the difficulties in characterizing and purifying macromolecular complexes. Nevertheless, several innovative strategies have been developed to deliver proteins intracellularly, and these delivery strategies provide a roadmap for developing intracellular protein therapeutics. In this review article we will discuss recent progress in 4 areas of intracellular protein therapeutics, which are focused on the following four classes of therapeutics, firstly, vaccine development, secondly, transcription factor therapies, thirdly, gene editing and finally, cancer therapeutics (Figure 1).

Intracellular protein delivery and vaccine development

Vaccines are arguably the most effective class of therapeutics ever developed. However, despite their success, vaccines still need to be developed for many pathogens, ranging from HIV to Hepatitis C. $CD8^+$ T cells play an essential role in combating infections generated from viruses and cancer and there is consequently great interest in developing vaccines that can efficiently activate $CD8^+$ T cells. A key step in developing vaccines that can activate CD8+ T cells is delivering antigen to the cytoplasm of antigen presenting cells (APCs). A large number of studies have now been published demonstrating that nanoparticles can deliver antigens into the cytoplasm of dendritic cells and activate CD8+ T cells. This is not surprising because the development of vaccines that can activate CD8+ T cells represents perhaps the most straightforward application of intracellular protein delivery [1], and is the most tolerant in terms of design parameters for developing delivery vehicles. For example, although protein antigens need to be delivered to the cytoplasm of dendritic cells to be presented as Class I antigens, the delivered antigens do not need to be folded, and can be denatured, as proteins processed for class I antigen presentation are degraded into peptides. Consequently, all of the problems associated with keeping proteins active and folded during the assembly of the

Figure 1



Protein therapeutics that function inside of cells: an emerging class of therapeutics. Overview of the four major applications of intracellular protein delivery: vaccine development, transcription factor therapy, gene editing and cancer therapy. Schematic diagram describing intracellular protein delivery. Green and blue items represent a cell and a protein therapeutic, respectively.

delivery vehicles are avoided. In addition, APCs and dendritic cells are phagocytic cells and robustly internalize particles between 100 nm-2 μm in size, which is fortuitous, because a variety of strategies exist for developing nanomaterials in this size range. Finally, class I antigen presentation can be quite efficient, and consequently, efficient CD8+ T cell activation can be observed, even if only a small fraction of antigen is delivered into the cytoplasm. A variety of elegant strategies have been developed for delivering antigens and adjuvants into the cytoplasm of dendritic cells [2–10]; Some of these are briefly summarized below.

Numerous microparticle strategies for delivering antigens into the cytoplasm have been developed. For example, Poly(lactic-co-glycolic acid) (PLGA) microparticles containing Ovalbumin, formulated via a double emulsion procedure, were able to efficiently deliver antigens and generate class I antigen presentation [11]. The mechanism by which PLGA can disrupt lysosomes is currently unknown, but presumably is related to hydrolysis of the ester linkage, which presumably causes colloid osmotic disruption of the endosome. A key benefit of the PLGA based systems is that the PLGA polymer itself has FDA approval for a variety of indications. In addition, the hydrolysis products of PLGA can themselves act as TLR ligands and danger signals, and this can further increase the efficiency of antigen presentation [12,13•]. However, the hydrolysis kinetics of PLGA are not perfectly tuned for the environment of the phagosome and consequently several other microparticle chemistries have therefore been developed for intracellular delivery of antigens.

Antigen loaded microparticles and microgels, made from acid degradable ketal linkages, can deliver antigens into the cytoplasm of APCs and generate Class I antigen presentation. Ketal linkages are ideal for cytoplasmic delivery because their hydrolysis rate is proportional to the hydronium ion concentration, and hydrolyze 250 times faster at the pH 5.0 environment of the phagosome, versus the pH 7.4 environment of the extracellular space [14,15•,16•,17]. Microgels made with ketals linkages should disrupt endosomes/phagosomes via a colloid osmotic mechanism because the hydrolysis of the ketal linkage will dramatically increase the solute concentration in the phagosome. In addition, ketal linkage hydrolysis can be exquisitely tuned and microgels with linkages have been developed that hydrolyze with a $t_{1/2}$ of several minutes at pH 5.0 and several hours at pH 7.4, which is ideal for intracellular drug delivery applications [17].

Transcription factor based therapeutics

Transcription factors (TFs) play a central role in biology, and are master regulators of biological function, through their ability to coordinate the expression of genes [18,19].