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Attenuated protein toxins as intracellular nucleic acid delivery fibromyalgia and chronic pain vectors

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Many protein toxins have evolved to access a variety of relatively inaccessible intracellular compartments in order to exert virulence. Counted among this number are proteins such as ricin toxin, shiga toxin, diphtheria toxin and anthrax toxin. These proteins display diverse architecture ranging from AB5 to AB configurations and depending upon the specific B chain in question, entertain a number of strategies from direct membrane penetration to utilizing retrograde trafficking pathways to access a plethora of intracellular compartments including the cytosol. Typically the A chain will exhibit catalytic activity proportional to both cellular intoxication and virulence. However given the facile nature of protein recombination, attenuation is relatively simple. Here we describe the ability of attenuated anthrax toxin (ATx) to manipulate endocytic cargo sorting for the purposes of drug delivery, traversing intracellular compartmental boundaries for nucleic acid delivery. We report not only the efficiency with which siRNA and antisense effectors are delivered but also the mechanisms they utilize to traverse the barriers responsible for intracellular compartmentalization. Attenuated Atx:ASO complexes had transfection efficiency approximately equivalent to Nucleofection®. In HeLa cells, at 200 pmol ASO expression of the target gene was 5.4±2.0% relative to an untreated control after 24 h. Using 200 pmol ASOs, Nucleofection® reduced Synt5 expression to 8.1±2.1% after 24 h. PA:LFn-GAL4:ASO transfection of non- or terminally-differentiated THP-1 cells and Vero cells resulted in 35.2±19.1%, 36.4±1.8% and 22.9±6.9% (respectively) target gene expression after treatment with 200 pmol of ASO and demonstrated versatility. Nucleofection® with Stealth RNAi™ siRNA reduced HeLa Synt5 levels to 4.6±6.1% whereas treatment with the PA:LFn-PKR:siRNA resulted in 8.5±3.4% Synt5 expression after 24 h (HeLa cells). These data underscore the tractability of this approach to both antisense and siRNA delivery.

Recent Publications

1. P D Dyer et al. (2016) An *in vitro* evaluation of epigallocatechin gallate (eGCG) as a biocompatible inhibitor of ricin toxin, Biochim. Biophys. Acta. 1860(7):1541-1550. Doi:10.1016/j.bbagen.2016.03.024.
2. P D Dyer et al. (2015) Disarmed anthrax toxin delivers antisense oligonucleotides and siRNA with high efficiency and low toxicity. Journal of Controlled Release. 220(PtA):316-328. Doi:10.1016/j.jconrel.2015.10.054.
3. S A Shorter et al. (2017) The potential of toxin-based drug delivery systems for enhanced nucleic acid therapeutic delivery. Expert Opinion on Drug Delivery. 14(5):685-696. Doi:10.1080/17425247.2016.1227781.
4. S A Shorter et al. (2017) Green fluorescent protein (GFP): is seeing believing and is that enough? Journal of Drug Targeting. 25(9-10):809-817. Doi:10.1080/1061186X.2017.1358725.
5. M W Pettit et al. (2014) Construction and physiochemical characterization of a multi-composite, potential oral vaccine delivery system (VDS). International Journal of Pharmaceutics. 468(1-2):264-271. Doi:10.1016/j.ijpharm.2014.03.046.

Biography

Simon C W Richardson is a Founder, Director and CSO at Intracellular Delivery Solutions Ltd, and Reader (Associate Professor) in Membrane Trafficking and Drug Delivery, at the University of Greenwich, UK. The driving theme behind his research is the intracellular delivery of antisense and RNAi to the cytosol. He is currently leading the Cell Biology Research Cluster within the Faculty of Engineering and Science, located at the Medway campus. His lab is currently working with several technologies based upon attenuated virulence factors that have very low *in vitro* toxicity profiles (and are minimally disruptive to the cell), and very high efficiency intracellular delivery profiles. We are also examining several methodologies to modulate protein stability and intracellular trafficking to aid the oral delivery of vaccines.

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