Challenges in cellular delivery of therapeutic proteins

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Abstract

Novel therapeutic approaches have been developed with the aim to replace the faulty gene or gene product in the diseased cells. Many strategies including viral and non-viral gene delivery vehicles have been developed for gene delivery but these are associated with safety issues such as insertional mutagenesis. From a therapeutic viewpoint, proteins are more advantageous as they have specific mechanisms of action and are highly potent. In addition as proteins do not cause alterations to the genome and are short-lived they should be safe and ideal for diseases such as cancer where long-term production is not necessary. However, the effectiveness of protein therapeutics is limited by several interrelated pharmaceutical issues including; in vitro and in vivo instability, insolubility when produced in eukaryotes, lack of appropriate posttranslational modifications, immunogenicity and shorter half-lives.

Modifications such as subtle changes in the protein structure or covalent attachment of moieties such as poly(ethylene glycol) (PEG), polysialic acid, or glycolic acid, as well as developing new formulations containing nanoparticulate, liposomes, polymeric microspheres, polymeric nanoparticles have been shown to improve the stability and efficacy of protein delivery. These strategies have the potential to be developed into next generation protein therapeutics.

We have been developing a therapeutic tumour selective pro-apoptotic protein system for in vivo and in vitro delivery. CAV-Apoptin is a protein from chicken anemia virus which has tumour selective cytotoxicity. Recently a novel human gyrovirus has been identified which encodes a homologue of Apoptin called HgyV-Ap which has 70% sequence homology with CAV-Apoptin in its functional domains and has similar anti-tumour function.

We have used the TAT-protein transduction domain to deliver both these proteins into a number of different cancer cell lines to test their therapeutic potential. Several modification to the TAT sequence as well as covalent attachment of poly (ethylene glycol) (PEG) and fusion to MBP were used to increase the solubility and efficiency of delivery of these proteins. The results obtained and the challenges faced will be discussed in the lecture.