# Efficient, non-genetic delivery of therapeutic proteins

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## Abstract

Due to their natural ability to deliver genes into animal cells viruses are the most efficient and commonly used eukaryotic delivery vehicles. However, there are a number of limitations to their routine therapeutic use including insertional mutagenesis, size limitation of the gene insert and possible immunogenicity.

The trans-acting activator of transcription (TAT) protein transduction domain (PTD) from human immunodeficiency virus type 1 mediates the transduction of peptides and proteins into target cells. The TAT-PTD has an important potential as a tool for the delivery of therapeutic agents. We have developed a protein delivery system by fusing several tumour specific pro-apoptotic genes to the TAT sequence for production of recombinant proteins. Both TAT-apoptin and TAT-E1A, two viral proteins with tumour selective cytotoxicity, were efficiently delivered into cancer and normal cells and induced apoptotis in tumours both in vitro and in xenograft tumour models of head and neck cancer. Furthermore, TAT-apoptin is being explored as an efficient strategy for ex-vivo purging of multiple myeloma cells from leukaemic cells for treatment by autologous bone marrow transplant. However, one drawback of this strategy is that the production of TAT fusion proteins in bacteria is associated with problems such as protein insolubility and the absence of major eukaryotic post-translational modifications. In addition, as a therapeutic tool the physical delivery of TAT fusion proteins such as direct injection into the tumour bed is inefficient as the recombinant proteins may not reach all the cells within the tumour mass. Furthermore, therapeutic delivery of proteins in vivo can result in the degradation by proteases and rapid elimination by renal filtration. Therefore multiple applications of the fusion proteins would be necessary.

An attractive alternative, both for in vitro protein production and for in vivo applications, is the use of higher eukaryotic cells for secretion of TAT fusion proteins. However, the ubiquitous expression of furin endoprotease (PACE or SPC1) in the Golgi/endoplasmic reticulum (ER), and the presence of furin recognition sequences within TAT-PTD, results in the cleavage and loss of the TAT-PTD domain during its secretory transition through the ER and Golgi. We have developed a synthetic peptide (TAT -PTD) in which mutation of the furin recognition sequences, but retention of protein transduction activity, allows secretion of recombinant proteins, followed by successful uptake of the modified protein by the target cells. This system was used to successfully secrete marker proteins and apoptin. Phosphorylation and induction of apoptosis by TAT -apoptin indicated that the secreted proteins were functional in target cells. In conclusion, the above protein delivery strategy offers a safe and efficient way to deliver eukaryotic proteins into cells and has important therapeutic potential.