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Endocytic DNA and siRNA delivery mediated by pH sensitive peptides

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Amphipathic peptides have emerged as promising candidates for both DNA and siRNA delivery. Consisting of both hydrophilic and hydrophobic domains, they bind to nucleic acids and at the same time provide pH dependent membrane destabilising activity, promoting endosomal escape. The aim of this study was to investigate the efficiency of such peptides in delivering siRNA and plasmid DNA, and to improve our understanding of how the structural difference between the peptides could affect the uptake mechanism and intracellular trafficking of the system. A series of structurally related histidine-rich amphipathic peptides (LAH4-L1, LAH6-X1L, LAH6-X1-26 and LAH6-X1-W) were investigated. The LAH peptides are 25–26 amino acids in length and comprise cationic lysines to allow electrostatic interaction and complexation with the negatively charged nucleic acids. Each of the peptides also contains four or six histidine residues. With a starting pK_a around 6.0, the imidazole group of histidine may allow buffering and subsequently destabilise endosomes, thus enhancing endosomal escape of the nucleic acids. The LAH peptides demonstrated pH responsive character which is classically manifested as a conversion from an alpha helical conformation at neutral pH to a disordered conformation at acidic pH. Differences in the number of charges and the hydrophobicity in the four peptides affect the nature and pH dependence of this transformation. Luciferase reporter gene studies showed that the *in vitro* DNA transfection efficiency of the LAH peptides were comparable to commercially available lipofectamine in both A549 and MCF-7 cells. These peptides, in particular LAH6-X1L, also showed high resistance to serum in MCF-7 cells. In addition, both LAH4-L1 and LAH6-X1L mediated significant knockdown of GAPDH enzyme in siRNA transfection studies in the presence of serum. Live cell confocal imaging

was carried out to study the intracellular trafficking of the peptide/nucleic acid complexes. Co-localisation experiments were performed with LAH6-X1L- DNA/siRNA complexes and dextran in A549 cells, with the nucleic acids labelled with rhodamine (red), nucleus labelled with Hoechst (blue) and dextran labelled with Alexa-fluor-488 (green). Dextran is known to be internalised through fluid-endocytosis and end up in endosomes and later lysosomes. At an early stage (within the first hour of post-transfection) there was a high level of co-localisation between LAH6-X1L complexes and dextran (shown as orange in colour). At later stages (300 minutes post-transfection), the degree of co-localisation significantly reduced as the siRNA (red) and dextran (green) was shown to be clearly separated from each other. Our results indicate that the histidine-rich peptides offer great promise as siRNA delivery vectors with the ability to promote endosomal/lysosomal escape.

doi:10.1016/j.drudis.2010.09.368

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Role of polymer architecture on polycation induced cell death: systematic study on molecular mechanism

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More than 3000 references can be found in the literature that relate to the application of polycations for drug or gene delivery. However, systematic studies that try to correlate polymer molecular weight, architecture and/or composition with polycation induced cell toxicity are scarce, and the underlying biomolecular mechanisms remain largely unknown. In this contribution new findings are presented on the mechanisms of polycation induced cell death and its correlation with the polymer architecture and degradation rate. For our studies, we firstly synthesized a polymer library based on L-lysine monomer units. The library contained linear, hyperbranched and dendritic L-lysine analogues in a broad range of molecular weights. We then investigated the effect of molecular weight (Mn), degree of branching and polydispersity on the mechanism of

short and long term cell toxicity *in vitro*. The molecular mechanisms underlying cell death at various stages of cell exposure to polycation were identified. The onset and extent of these specific modes of cell death were shown to be dependent on the size and degree of branching of the polycations. Simultaneously the *in vitro* degradation profile for analogues was assessed and correlated with the process of cell death. For the first time the factors contributing to the differential toxicity profile of the L-lysine analogues are analyzed and discussed.

doi:10.1016/j.drudis.2010.09.369

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A novel 3D model for the study of functionalised-nanoparticle penetration into human tissue

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The advancing field of nanotechnology is progressing rapidly towards the development of multifunctional nanoparticles for use in biomedicine. These nanoparticles benefit from functional biomolecules attached to their surface and can act as unique carrier systems. However, the impermeable nature of both the plasma and nuclear membranes hinders their potential. Two current methods to enhance uptake are using external magnetic fields to remotely control particle direction, and functionalising the nanoparticles with a cell penetrating peptide; both of which facilitate cell entry. To date, studies have largely adopted traditional 2D cell monolayers, the results of which cannot reliably be translated to a human body. This study has focused on using 3D collagen gels seeded with human fibroblast cells as a tissue equivalent model for the study of nanoparticle penetration into human tissue. Iron oxide nanoparticles were employed, which have an attached cell penetrating peptide (penetratin); are magnetic (to allow external control via magnetic fields); and are fluorescent (to allow visualisation). Various analytical techniques were used including fluorescence staining, TEM and histology to compare nanoparticle penetration into gel models both with/without penetratin attachment, and with/without the presence of a magnetic field; both of which have previously been shown to increase nanoparticle uptake in monolayer cul-