we studied the optimal conditions for attachment and proliferation of the astrocytes and hBECs. Furthermore, we monitored the effect of hBEC growing directly on the surface of an adherent astrocytic monolayer. The tight junctions between the brain endothelial cells forms a diffusion barrier that is responsible for the high paracellular resistance which is a crucial characteristic for any B3-model. In order to test the integrity of this barrier in the B3-model and simultaneously measure the transcellular transport we combined fluorescent compounds and dye labelled large molecules to test the permeability across the barrier. This strategy allows for the discrimination between transcellular and paracellular transport.

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Solid lipid nanoparticles for gene delivery into prostate cancer cells

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Prostate adenocarcinoma is the most common cancer occurring in male. The aim of this study is to develop a gene delivery system based on solid lipid nanoparticles (SLNs) for the transfer of tumor suppressor genes that are able to induce death into prostate cancer cells. Formulations of cationic SLNs, consisting of stearic acid/DOTAP/pluronic, were produced. Additionally, formulations with and without 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) in various molar ratios were tested. The SLNs produced were approximately 100 nm in size and showed a positive surface charge (+40 mV) in water. The SLNs showed excellent stability, as evidenced by size, zeta potential, transfection efficiency over 140 days, and possibility of lyophilization and/or sterilization without loss of efficiency. The SLNs were able to protect genetic material against DNase digestion and showed a transfection capacity comparable to that of Lipofectamine 2000®, a commercially available gene carrier. Interestingly, we found that the transfection efficiency of SLNs in prostate cancer PC3 cells was significantly higher when compared to that in normal human prostate PNT2-C2 cells. Further examination revealed that this is due to enhanced endosomal escape rather than enhanced internalization of SLNs in prostate cancer cells. These results indicate that cationic SLNs are a promising tool for gene delivery into prostate cancer cells.

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Kinases in cationic lipid/polymer-mediated gene delivery

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Cationic lipids/polymers, complexed with DNA (also called lipo/polyplexes), are promising tools for gene delivery or transfection. Lipo/polyplexes have low toxicity, a relative low immunological response and can be synthesized on large scale. Lipo/polyplexes are internalized by cells via endocytosis. The endocytotic pathway that is used by lipo/polyplexes depends on the cell type and the type of lipo/polyplexes, and likely contributes to transfection efficiency. We have recently shown that adhesion receptors are involved in binding and endocytosis of lipoplexes. Cell receptors also have been described for the endocytosis of polyplexes. Receptor occupation can initiate signaling cascades, commonly mediated by kinases, which in turn tightly regulate endocytosis and endocytotic processing. The elucidation of cellular signaling signatures, initiated by lipo/polyplexes and/or those that allow or preclude gene delivery, will be instrumental in understanding the interaction between lipo/polyplexes and cells at the molecular level and contribute to the design of protocols with improved gene delivery efficiency. In this study we have performed a screen with a wide range of validated pharmacological kinase inhibitors, and evaluated their effects on lipo/polyplex transfection efficiency. In this screen a kinase is identified that specifically influences the transfection efficiency of a polyplex. It is further demonstrated that, as a part of the underlying mechanism, this kinase regulates the endocytotic processing of the polyplex and, as a consequence, controls its endosomal escape.

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Peptide-based nano-particle for *in vivo* delivery of siRNA

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The development of short interfering RNA (siRNA), has provided great hope for therapeutic targeting of specific genes responsible of pathological disorders. However their clinical application remains limited by their poor cellular uptake, low bioavailability, and insufficient capability to reach targets *in vivo*. We have designed a novel approach, based on short amphipathic peptides 'CADY' that promotes efficient delivery of siRNA into wide variety of mammalian cell lines and *in vivo* upon systemic and topical administrations. This carrier consisting of a balance between hydrophobic and hydrophilic domains and forms stable discrete 'nanoparticles' with siRNA, through non-covalent interactions. Cellular uptake mechanism of CADY/siRNA nanoparticles is dependent on the size of the particle and involves membrane potential and dynamic, which enables a rapid release of the siRNA into the cytoplasm and promotes a robust down-regulation of target mRNA. CADY-carriers were applied to the delivery of siRNA targeting the cell cycle regulatory protein Cyclin B1 into cancer cells. We demonstrated that when associated with CADY, sub-nanomolar concentrations of siRNA Cyclin B1 significantly knocked down Cyclin B1 protein levels resulting in cell cycle arrest in G2 arrest and blocked cancer cell proliferation. The surface of CADY particles can be functionalized and addition of cholesterol-moiety significantly improves siRNA stability *in vivo*, thereby enhancing the efficiency of this technology for systemic administration following intravenous injection. We have validated the therapeutic potential of this strategy for cancer treatment by targeting cyclin B1 in various mouse tumour models and demonstrate that CADY-mediated delivery of cyclin B1 siRNA prevents tumour growth *in vivo* following systemic intravenous injection. Moreover, we showed that functionalization of CADY particles with other chemical groups or biological moieties can be applied to generate formulations to target specific cell types or tissues which can