1080. Receptor-Targeting Smart Vectors for Efficient Gene Transfer to Tumours

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We seek to develop novel synthetic vector formulations that can be administered systemically to target therapeutic genes to tumours. Cationic nonviral vectors are cleared rapidly from the circulation by the reticuloendothelial system as a result of binding to plasma proteins and vector aggregation. Circulation times may be extended by shielding the vectors with polyethylene glycol (PEG) moieties. However, PEGylation often leads to greatly reduced transfection efficiency due to excessive vector stability. The aim of this project was to develop novel formulations of PEGylated vectors which may be administered intravenously (i. v.), and persist in the circulation and target tumour-associated receptors. The vector has been further modified to disassemble within the cell in response to the intracellular environment to achieve high transfection efficiency. Such virus-like vector formulations are often referred to as "smart vectors".

The vector developed is a lipopolyplex-class formulation based on an integrin-targeting peptide with an oligolysine nucleic acid-binding element, and a cationic lipid that enhances integrin-targeted transfection by promoting endosomal release. New cleavable peptides (CP) were designed containing CP motifs located between the DNA-binding and the integrin-targeting domains. Peptide cleavage should enhance transfection by promoting the trafficking of DNA to the cytoplasm and so to the nucleus. Cleavable PEGylated lipids (PEG-CL) were also synthesised for improved *in vivo* stability in blood, enhancing intracellular complex dissociation and endosomal escape.

The cleavage studies showed PEG-CL were efficiently hydrolysed at acid pH using TLC, and CP were cleaved in the presence of endosomal proteases using HPLC. The size of lipopolyplexes comprising PEG-CL in physiological salt solution was 394±14 nm by light scattering methods, and stable for at least one hour, which was considerably smaller ($p<0.05$) than complexes made of unPEGylated lipids (1388±198 nm after one hour). The smaller particles generated with PEG-CL were less efficient in *in vitro* transfections unless a centrifugation (1500 rpm, 5 min) was performed to promote their sedimentation and cell contact. After centrifugation, in murine neuroblastoma cell line (Neuro2A), transfection efficiency with lipopolyplexes containing PEG-CL, CP and a plasmid encoding the luciferase gene was 2 times more efficient than with Lipofectin and non-CP ($p<0.05$). The transfection efficiency of PEG-CL/CP lipopolyplexes using a plasmid encoding the GFP protein, was about 40% in Neuro2A and mouse fibroblast (AJ3.1) cells, whereas only 20% of these cells were transfected using Lipofectin and non-CP ($p<0.05$). Finally, in a murine neuroblastoma model, i. v. administration of these integrin-targeted PEG-CL/CP lipopolyplexes showed a higher level of luciferase expression in tumour (52142±7121 RLU/mg) than in lung (2347±577 RLU/mg) or in liver (1198±58 RLU/mg) ($p<0.05$).

In conclusion, we have developed a novel, targeted, synthetic, smart vector formulation that offers exciting prospects for tumour-specific therapeutic gene transfer.