393. Efficient Gene Transfer with Receptor-Targeting Vectors for Immunotherapy of Tumours

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The development of non-viral vectors that can be administered systemically to target therapeutic genes to tumours is a major challenge since cationic vector particles are cleared rapidly from the circulation due to vector aggregation and binding to plasma proteins. These problems may be overcome by shielding the vectors with polyethylene glycol (PEG) moieties, however, this often leads to loss of transfection efficiency. To solve this problem we have designed a self-assembling formulation of PEGylated cationic liposomes and a cationic targeting peptide which both contain chemical linkers that cleave in response to the endosomal/lysosomal environment, removing the PEG chain and promoting vector disassembly. The cationic liposome (ME42) contains a short PEG sequence of four repeat units attached to the cationic headgroup *via* a cleavable ester linkage. The peptide contains an RGD integrin targeting motif, linked to a sixteen-lysine DNA-binding sequence via an RVRR peptide motif, cleavable by endosomal enzymes. The lipid and peptide components self-assemble upon mixing with plasmid DNA into spherical, receptor-targeted nanocomplexes (RTNs) of 50 to 100 nm, as assessed by electron microscopy. *In vitro* experiments demonstrated esterase-mediated cleavage of the lipid, and cathepsin B-mediated cleavage of the peptide, both as free reagents and in RTNs. It was then shown that the cleavable RTN formulation retained higher transfection efficiencies in a number of cultured cell types than the non-cleavable complexes. *In vivo* studies were performed in A/J mice engrafted with Neuro-2A cells subcutaneously, a widely used model of neuroblastoma. RTN formulations, with the luciferase reporter gene injected into the mouse tail vein generated high levels of expression in tumours in >90% of mice with little expression in lung, liver, spleen and other organs. Efficiency of delivery was greater in targeted RTNs compared to non-targeted nanocomplexes and higher in cleavable RTN formulations compared to non-cleavable formulations. Uptake into the tumour may be mediated by the enhanced permeation and retention (EPR) effect, supported by the observation that the distribution of expression throughout each tumour varied. Expression in some parts was as high as 60% of tumour cells while there was little in others, and this was supported by luciferase data. The cytokines interleukin-2 (IL-2) and IL-12 were shown previously to enhance the immune response against established neuroblastoma tumours in mice. We have now shown that genes encoding these cytokines administered in the cleavable RTN formulation by multiple tail vein injections effectively retarded tumour growth with survival increased up to 3-fold. Expression of both cytokines was quantified by ELISA in tumours, while infiltrating leukocytes were greatly increased in the treated mice, assessed by flow cytometry, compared to controls, indicating an immune response against the tumour. In conclusion, we have developed a novel, targeted, fully-synthetic, smart vector formulation that offers exciting prospects for tumour-specific therapeutic gene transfer.