Targeted non-viral delivery of mini-intronic plasmids for rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is an autoimmune disorder of the joints characterised by inflammation, synovial hyperplasia and increased vascularisation. Synovial fibroblasts are aggressive cells in RA pathogenesis, contributing to inflammation and cartilage degradation. Current treatment with disease modifying anti-rheumatic drugs increases risk of serious infection. Further, some patients do not respond to drugs whilst others develop resistance leading to loss of efficacy. Hence, we aim to develop a gene therapy that modulates RA disease without life-threatening, systemic immunosuppression.

Our approach involves developing receptor-targeted nanoparticles (RTN) that selectively deliver therapeutic genes to the inflamed synovium, thereby improving efficacy and reducing systemic side-effects. The RTN comprises of a cationic lipid that self-assembles with the anionic DNA backbone and a neutral lipid DOPE to aid DNA endosomal escape. In addition, formulations include a peptide containing a cationic, 16-lysine domain for electrostatic DNA packaging and a synoviocyte targeting ligand, separated by a cleavable or hydrophobic linker to alter RTN stability. Further, we aim to develop a DNA vector that provides high and sustained therapeutic gene expression while minimising inflammatory response based on mini-intronic plasmids (MIPs). MIPs lack bacterial propagation sequences, making them safer and potentially less inflammatory, and have been shown to provide greater and more prolonged gene expression than conventional plasmids.

Methods: Rabbit synovial fibroblasts (HIG-82) were transfected with luciferase or GFP reporter plasmids with RTNs containing different peptides. Transfections to assess cell type specificity were performed with chondrocytes (C28/I2) or hepatocytes (HepG2). Luciferase or GFP expression was measured after 24 or 48-hours incubation, respectively. MIPs contained a luciferase reporter gene with either a CMV or EF1α promoter and luciferase activity following HIG-82 or HEK293T transfection with RTNs was compared to that of conventional plasmids over time at equimolar ratios.

Results: RTNs with synoviocyte peptides demonstrated equal transfection efficiency in HIG-82 cells than positive control peptides that have previously shown good efficiency in various cell types, regardless of linker type. In comparison, synovial-targeted RTNs yielded much poorer transfection efficiency in hepatocytes and chondrocytes compared to RTNs with positive control peptides, which transfected all cell types indicating a degree of targeting specificity for synoviocytes. Additionally, RTNs with cleavable peptides gave more efficient transfection than hydrophobic in all cell types, presumably due to enhanced RTN disassembly following cell uptake.

When MIPs were delivered with RTNs to HIG-82 or HEK293T cells there was no clear difference in transfection efficiency as compared to conventional plasmids. Additionally, it was predicted that MIPs may be less toxic than conventional plasmids due to their lack of bacterial CpG sequences and their smaller size allowing for a lower amount of DNA to achieve the same gene copy number. However, no difference in cytotoxicity was noted between MIPs or conventional plasmids in either HIG-82 or HEK293T cells. Investigations are ongoing to find a suitable vector that provides the required sustained expression.

Conclusions: This work provides the basis for a targeted RA gene therapy approach that specifically delivers DNA to synoviocytes in the inflamed joint.