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Non-viral dumbbell-shaped DNA vectors for efficient delivery and expression of recombinant genes in human tissue culture and primary cells

Volker Patzel
National University of Singapore, Singapore

Statement of the Problem: Various therapeutic approaches depend on efficient delivery of recombinant DNA into target cells. To facilitate DNA delivery, researchers use viral or non-viral vectors. However, most of the existing vector systems harbor severe safety risks and/or suffer from low delivery efficiencies.

Methodology & Theoretical Orientation: We designed and tested novel minimalistic dumbbell-shaped DNA vectors and developed novel methods for dumbbell vector production. Minimized non-coding RNA expressing dumbbells were only 130 bp in size representing the smallest expression vectors ever reported. To facilitate cellular dumbbell delivery, we covalently linked cell penetrating peptides (CPPs) to the dumbbell vectors.

Findings: Our new protocol produced dumbbell vectors more rapidly with higher yields and purity and at lower costs compared with conventional strategies. Rationally designed dumbbell vectors exhibited facilitated kinetics of cellular delivery, nuclear uptake, transcriptional activity and biological activity. Dumbbell-driven gene expression was enhanced more than 100 fold as compared with plasmids and not silenced in human primary cells. In primary human T cells, miRNA expressing dumbbell vectors were found to be more stable and active than plasmids or miRNA mimics. CRISPR/Cas-expressing dumbbell vectors were more potent than corresponding plasmid vectors. CPP/shRNA-dumbbell conjugates were efficiently taken up by human tissue culture cells and triggered significant target gene knockdown. Currently we are exploring dumbbell vectors in a trans-splicing based suicide gene therapy approach, for somatic cell reprogramming and for CRISPR/Cas genome editing.

Conclusion & Significance: Non-viral non-integrating dumbbell vectors to trigger profound, prolonged expression of recombinant genes in human primary cells. Thus, dumbbells are suitable for therapeutic applications that require transient expression in primary cells but in which vector integration is neither required nor desirable. These applications include RNA-guided genome editing, somatic cell reprogramming, suicide gene therapy and genetic vaccination. Our findings suggest dumbbell vectors represent a useful tool for regenerative medicine.

micvp@nus.edu.sg