Design of Minimal Dumbbell-Shaped DNA Vectors for Small Non-Coding RNA Expression

Jiang Xiaou

Abstract
A major barrier of using non-viral vectors for gene therapy is the short duration of transgene expression in postmitotic tissues. Previous studies showed transgene expression from conventional plasmid fell to sub-therapeutic level shortly after delivery even though the vector DNA was retained, suggesting transcription was silenced in vivo. Emerging evidence indicates that plasmid bacterial backbone sequence is responsible for the transcriptional repression and this process is independent of CpG methylation. Dumbbell-shaped vectors consisting solely of essential elements for transgene expression (promoter, transcriptional terminator, coding genes, RNA stabilizing sequences etc.) have been developed to circumvent these drawbacks. This novel non-viral vector system has been shown to improve transgene expression in vitro and in vivo. An impressive safety profile has also been obtained from one clinical trial using a dumbbell-shaped vector, demonstrating that the vectors are non-toxic and non-inflammatory (ClinicalTrials.gov NCT02077868).

We have designed dumbbell-shaped vectors for small RNA expression. The production procedure has been optimized, and the efficacy has been tested in tissue culture cells and human activated primary T cells. Some novel molecular features have been investigated and are shown to either improve the biological activity of the vector or ease the production. This may facilitate the development of dumbbell-shaped vectors for both preclinical investigation and gene therapy for human diseases.

Biography
Xiaou received his bachelor’s degree for biotechnology in College of Life Sciences, Zhejiang University in 2010. He subsequently received his PhD in biotechnology in NUS under the supervision of Dr. Volker Patzel in 2015. Currently he works as a Research Fellow in Dr. Patzel’s group. His research focuses on the development of non-viral vectors for therapeutic applications and small non-coding RNA biology.

Anti-HLA Alloantibodies as Quality Control Reagents for Clinical Diagnostic Platform

Gu Yue

Abstract
Antibodies targeting human leukocyte antigens (HLAs) is proposed to be one of the leading causes for chronic transplant rejection. However, alloantibodies have also been detected in some long-term graft survivors. One popular hypothesis that explains this paradox is that some HLA epitopes may be more immunogenic or some alloantibodies more pathogenic than others, and thus more likely to cause graft rejection. As such, there remains an urgent need for diagnostic and prognostic assay that can better predict the likelihood of antibody-mediated rejection responses taking place. We have generated and characterized fully human monoclonal IgG1 alloantibodies that have illustrated binding activities against HLA-A. One of the potential applications of these antibodies is to serve as the quality control reagents for new clinical diagnostic assays. Testing of selected alloantibodies on the current diagnostic platform has revealed an unexpected discrepancy from our laboratory results, suggesting that the clinical alloantibody detection reagents we tested may produce inconsistent and unreliable readings.

Biography
Gu Yue received her Bachelor’s degree in Life Sciences from NUS in 2015 before joining Associate Professor Paul A. MacArty’s lab as a PhD student. She is currently working on the generation and characterization of anti-HLA alloantibodies as well as exploring their roles in solid organ transplantation rejection.