Abstract of Doctoral Dissertation

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Design and *In vivo* Evaluation of Novel mRNA Lipid Nanoparticles Beyond the Hepatocytes Towards a New Era of Personalized Gene Therapies

(肝実質細胞を越える mRNA 搭載脂質ナノ粒子の設計と in vivo 評価による個別化遺伝子治療の幕開け)

RNA therapeutics are currently at an inflection point and recent progress in genetic drugs has paved the way for the development of a broader second wave of therapies. RNA drugs hold real potential for tackling devastating diseases that are currently resistant to small molecule drugs or monoclonal antibodies. Messenger RNAs (mRNAs) are an emerging therapeutic modality that holds great promise to treat genetic disease specifically and completely. mRNA has been used as a vaccine, as a protein replacement therapy, and even as a means of inducing permanent genomic editing via CRISPR. However, the clinical realization of RNA drugs is limited by the design of safe, targeted, cost-effective and efficient delivery technologies.

Furthermore, the design of non-viral vectors beyond hepatocytes for other targets without targeting ligands continues to be an elusive dream. Despite the known concept that active targeting could be achieved by suitably decorating nanoparticles with a specific targeting ligand, we herein provide a demonstration of the surprising ability of a class of engineered ionizable lipo-polyether and lipo-polyether-RNA transporters ($iPORTs_{ethers}$ and $iPORTs_{esters}$) based on living/controlled ring-opening polymerization of lactone and epoxide monomers that could circumvent the liver to efficiently and yet selectively deliver mRNA to the lungs and immune cells in spleen without the need for targeting ligands after systemic administration. We here in report a new series of materials that can achieve safe, efficient and targeted mRNA delivery to Antigen Presenting Cells (APCs) without the need for molecular or active targeting with strong potential for translating mRNA cancer vaccines for the next stage. Our top performing iPORT based LNPs elicited strong antitumor activity both in a prophylactic and therapeutic approaches. By the virtue of biosafety, efficiency and targeting, we believe that Estriol-GA05-30 LNPs have strong potential for translating mRNA cancer vaccines for the next stage. In another hand, our $\mathcal{E}$-decalactone based lipomers NP technology enabled selective

and efficient mRNA delivery to tissues beyond the hepatocytes. We show that expanding the chemical space of smart materials could enable the directed evolution of NPs for hard-to-reach targets without the need for targeting ligand. In particular we identified lipomers which elicited efficient mRNA expression *in vitro* and *in vivo*. The engineered $\mathcal{E}$-decalactone lipomers reported herein represent a versatile approach for the simple engineering of efficient lung targeted gene therapies from renewable sources. Furthermore, diversifying the chemical space of $\mathcal{E}$-decalactone lipomers via design of combinatorial libraries could be an easy and scalable strategy for development of the next generation gene therapies beyond the hepatocytes.

Optimization of the monomeric structure was proven to be a novel strategy to improve mRNA delivery efficiency and control the in vivo tropism. Interestingly, co-assembly of iPORTs with phospholipids and cholesterol can open new avenues for endless space of optimization that could be exploited for achieving interesting non-hepatocyte RNA delivery. Controlling the tail length of iPORTs (DP) was shown a key factor to enhance the efficiency of RNA delivery where it was initiator dependent and required case by case optimization. We believe that iPORTs technology could pave the way for clinical translation of mRNA drugs to treat lung diseases and offer endless possibilities to engineer the immune cells in spleen. This would interestingly help the wide range application and affordability of the advanced gene therapies for patients suffering from hard-to-treat diseases such as cancer, autoimmunity, and infectious diseases. This cost-effective, simple design based on commercially available monomers and monomers coming from sustainable sources could be an efficient strategy for evading *in vivo* barriers and successfully delivering gene therapies to hard-to-reach target sites without targeting ligands to treat undruggable diseases such as cancer, autoimmunity, and genetic diseases.