学 位 論 文 の 要 約

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学 位 論 文 題 名

Extrahepatic gene delivery for cancer treatment and
an investigation of factors that affect *in vivo* functional gene delivery
（肝臓外組織への遺伝子送達及び機能的遺伝子送達に影響する因子の探索）

The era of nucleic acid nanomedicine has arrived, as evidenced by the advent of Patisiran, the small interfering RNA (siRNA) encapsulated lipid nanoparticle (LNP), and the recently developed messenger RNA (mRNA) vaccines for COVID-19, an mRNA-loaded LNP. The diversity of nano-designs and the delivery of different siRNA, microRNA (miRNA) and DNA molecules in Phase II/III clinical trials reflects the potential of these nano-drug delivery system technologies. These breakthroughs in the field of non-viral gene delivery have attracted substantial interest worldwide and clearly point to the importance of non-viral systems such as LNPs for developing more effective drugs in the future. However, there are several challenges that delay the development of LNP-based gene therapy, such as extrahepatic gene delivery (I), the conventional screening methods that take time and cost (II), and unknown gene delivery mechanisms (III).

This work mainly addressed these three challenges, and demonstrated that LNPs can target an extrahepatic tissue for tweaking their lipid compositions without any of targeting ligands. The difference in tissue/cell-selectivity in the functional gene delivery of LNP deliveries was principally arise from the difference in intracellular processes, especially in the translation step. Specifically, Chapter 2 presented pDNA delivery to splenic immune cells by tuning lipid compositions of LNPs, and its application to DNA vaccinations for cancer treatment. The spleen-selective LNPs were mainly taken up by B cells and macrophages in the spleen, and B cells played a dominant role in the anti-tumor immune response induced by the LNPs.

In Chapter 3, we designed the transcribed pDNA-barcode for high-throughput *in vivo* screening of a variety of LNPs prepared with a novel lipid library, DOPE-derivative lipids. According to *in vitro* transfection experiments of different LNPs and *in vivo* analysis of functional gene delivery by utilizing the pDNA barcoding, a diverse set of chemically modified DOPE phospholipids and their combinations with different ionizable lipids can shift the transfection efficiency and the *in vivo* functional delivery of pDNA. Importantly, our results showed the poor correlation between cellular uptake/biodistribution and functional delivery (RNA/protein expression), indicating that intracellular events have a greater impact on the functional gene delivery than cellular uptake.

Chapter 4 aimed to elucidate the mechanisms driving differences in gene expression of delivered nucleic acids by comparing two types of LNPs which have different tissue-tropism of pDNA delivery, one being liver-selective and the other spleen-selective. First, gene expression and biodistribution of each LNP was measured after the injection via tail vein into mice. We observed little difference in the biodistribution of the two LNPs despite the 100~1000-fold difference in gene expression. Next, we quantified the amount of pDNA and mRNA in each tissue both intracellulary and in the nucleus by qRT-PCR in order to evaluate the intracellular processes, such as nuclear delivery, transcription and translation. These results showed more than 100-fold difference in the translation step while there were little differences in nuclear delivery of pDNA or the amount of mRNA expression between two LNP deliveries. These quantitative analysis indicate that biodistribution is not sufficient for evaluation of functional gene delivery, and it is necessary to determine where the LNP transfection affects its biological process influencing protein expression such as translational process on the cell. Furthermore, by using Apoe-/- mice and conducting CD21/35 blocking experiments, we showed that endogenous factors influenced tissue-selectivity of *in vivo* functional gene delivery as well as the biodistribution; the liver-selective LNPs produced a high gene expression in the liver by ApoE-dependent mechanisms, the spleen-selective LNPs showed a high gene expression in the spleen via complement-mediated uptake pathways.

Taken together, the data presented in this work provide an important framework: LNP components alter the formation of the protein corona and this affects uptake pathways and cellular states thus influencing functional gene delivery as well as biodistribution.