Department of Advanced Nanomedical Engineering

Professor Satoshi Uchida
Lecturer Yuki Mochida

Messenger RNA (mRNA) vaccines have proven highly effective and safe against COVID-19, with billions of doses administered to humans within just one year of emergency approval. This success has spurred intensive research and development in mRNA vaccines and therapeutics across various medical fields. These include infectious disease vaccines, cancer vaccines and immunotherapy, treatments for single-gene disorders, regenerative medicines, and genome editing. These applications require nano-sized drug delivery systems (nano DDS) for targeted mRNA delivery to specific tissues and cells. Our research focuses broadly on the nano DDS development and its therapeutic applications.

1. Nano DDS development

Our nano DDS utilizes polymeric micelles (PMs) coated with poly(ethylene glycol) (PEG). PMs prevent mRNA recognition by innate immune receptors in cells (**Fig. 1**), enabling mRNA delivery with minimal inflammatory responses. However, enhancing nuclease stability remains a challenge for PMs.

Current nano DDS development primarily concentrate on designing lipids and polymers. Alternatively, Prof. Uchida has pioneered nano DDS development centered on mRNA designs. Conventional methods directly modify mRNA molecules, for example by pseudouridine. Nonetheless, this strategy can compromise mRNA translational activity, limiting modification options. In contrast, our mRNA engineering approach involves hybridizing mRNA with RNA oligonucleotides bearing chemical moieties that stabilize nano DDS (**Fig. 2**). This strategy successfully enhances PM stability by introducing cholesterol moieties to mRNA, crosslinking mRNA and polycations in environment-responsive manners, and providing mRNA with steric structures (***Adv Drug Deliv Rev*** 199, 114972, (2023)).

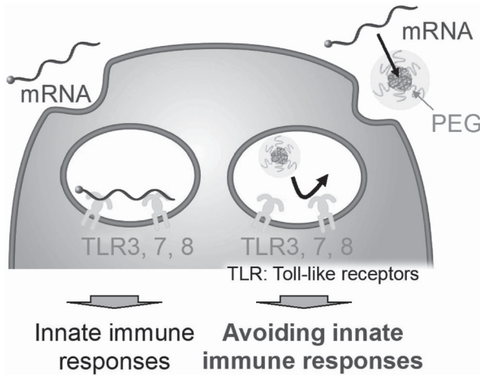

Fig. 1 Polymeric micelles

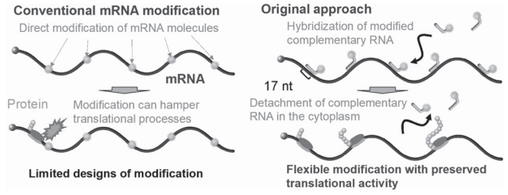

Fig. 2 mRNA engineering

2. Applications of mRNA vaccines and therapeutics

1) Infectious disease vaccines

A challenge of current mRNA vaccines based on lipid nanoparticles (LNPs) is their relatively high reactogenicity. While such reactogenicity is acceptable for a few doses in pandemic, safer vaccine platforms are needed for repeated boosting against COVID-19 and for other infectious diseases. To address this, we are developing carrier-free naked mRNA vaccines. For efficient vaccination using naked mRNA, we targeted the dermal tissue rich in antigen-presenting cells and used a jet injector to improve the delivery efficiency. This strategy achieved efficient antibody production and protective effects against SARS-CoV-2 challenge in mice, without systemic adverse effects. This vaccine was also effective in non-human primates.

2) Cancer vaccines

Unlike infections disease vaccines that require safety improvement, the primary challenge with cancer vaccines is enhancing their efficacy. The challenge stems from the low antigenicity of self-derived tumor antigens and the immunosuppressive tumor microenvironment. To boost vaccination efficacy, we employ mRNA engineering, introducing immunostimulatory adjuvant functionalities to mRNA (***PNAS*** 120, e2214320120, (2023)). By hybridizing mRNA with immunostimulatory double-stranded RNA (dsRNA) targeting RIG-I, an innate immune receptor, we prepared comb-structured mRNA (**Fig. 3**). This approach enables controlling immunostimulation intensity by adjusting the number of dsRNA strands, preventing excessive inflammatory responses. In mouse experiments, the comb-structured mRNA improved antigen-specific cytotoxic T lymphocyte (CTL) activity of lipid-based systems used in clinical trials, showing efficient anti-cancer responses against melanoma by targeting a tumor-associated antigen. This system also enhanced the CTL responses of other vaccine delivery systems, such as PM and LNP used in an approved COVID-19 vaccine.

3) Protein replacement therapy and genome editing

Apart from vaccine applications, mRNA offers promise in expressing therapeutic proteins and inducing genome editing. In these applications, immunostimulation induced by mRNA delivery systems can cause adverse effects and hinder disease treatment in the target tissue. Here, the low immunostimulatory properties of PMs is beneficial. Indeed, using PMs, in animal models, we succeeded in treating fulminant hepatitis using anti-apoptotic mRNA, brain ischemia and spinal cord injury using neurotrophic factor mRNA, Alzheimer's disease using mRNA encoding single-chain antibody against amyloid β, and osteoarthritis and intervertebral disc disease using chondrogenic mRNA. Moreover, RNA-based delivery of CRISPR/Cas9 using PMs has effectively induced in vivo genome editing in the mouse brain.

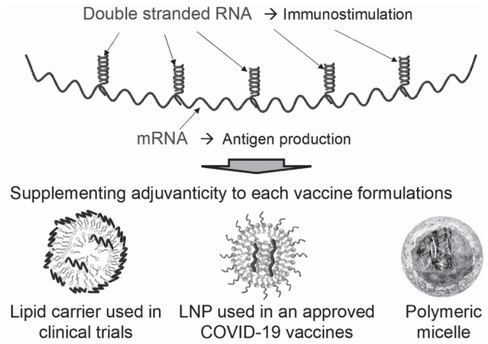

Fig. 3 Comb-structured mRNA for cancer vaccines

Publications

M Inagaki, N Abe, Z Li, Y Nakashima, S Acharyya, K Ogawa, D Kawaguchi, H Hiraoka, A Banno, Z Meng, M Tada, T Ishida, P Lyu, K Kokubo, H Murase, F Hashiya, Y Kimura, S Uchida, H Abe, Cap analogs with a hydrophobic photocleavable tag enable facile purification of fully capped mRNA with various cap structures, ***Nature communications*** 14, 2657, (2023)

T A Tockary, S Abbasi, M Matsui-Masai, A Hayashi, N Yoshinaga, E Boonstra, Z Wang, S Fukushima, K Kataoka, S Uchida, Comb-structured mRNA vaccine tethered with short double-stranded RNA adjuvants maximizes cellular immunity for cancer treatment, ***Proceedings of the National Academy of Sciences*** 120, e2214320120, (2023)

M Oba, M Shibuya, Y Yamaberi, H Yokoo, S Uchida, A Ueda, M Tanaka, An Amphipathic Structure of a Dipropylglycine-Containing Helical Peptide with Sufficient Length Enables Safe and Effective Intracellular siRNA Delivery, ***Chemical and Pharmaceutical Bulletin*** 71, 250-256, (2023)

W Yang, T Miyazaki, Y Nakagawa, E Boonstra, K Masuda, Y Nakashima, P Chen, L Mixich, K Barthelmes, A Matsumoto, P Mi, S Uchida, H Cabral, Block catiomers with flanking hydrolyzable tyrosinate groups enhance in vivo mRNA delivery via pi-pi stacking-assisted micellar assembly, ***Sci Technol Adv Mater*** 24, 2170164, (2023)