Title	Development of A Nanostructured RNA/DNA Adjuvant Targeting Toll-like Receptor 7/8
Keywords (up to 5)	RNA, TLR7/8, adjuvant
Authors	Fusae Komura[1], Makiya Nishikawa[2], Yuki Takahashi[1], Yoshinobu Takakura[1] [1]Graduate School of Pharmaceutical Sciences, Kyoto University, Japan [2]Faculty of Pharmaceutical Sciences, Tokyo University of Science, Japan
Abstract	Single-stranded RNA (ssRNA) is recognized by Toll-like receptor 7 and/or Toll-like receptor 8 (TLR7/8) and induces high immune response. Under in vivo conditions, RNA is too unstable to be delivered to immune cells that express TLR7/8, so that RNA needs to be stabilized by any means, including chemical modification and complex formulation with phospholipids, for in vivo application. Our previous study showed that polypod-like structured DNA is an effective system to deliver immunostimulatory CpG DNA to immune cells.[1, 2] In this study, we aimed to develop a nanostructured RNA/DNA adjuvant targeting TLR7/8. Nanostructured RNA/DNA assembly was designed using a 40-mer oligoribonucleotide and three 40-mer oligodeoxynucleotides. Electrophoresis showed that the tetrapod-like RNA/DNA nanostructure was successfully formed with high yield. The RNA/DNA nanostructure was more stable than ssRNA under 10% fetal bovine serum (FBS) conditions. The target TLRs were identified using HEK-Blue human TLRs cells by measuring secreted alkaline phosphatase (SEAP) activity. SEAP assay showed that the RNA/DNA nanostructure triggered human TLR8-specific responses. The uptake by immune cells was examined using the RNA/DNA nanostructure prepared using 5-6-carboxyfluorescein (FAM)-labeled RNA. Flow cytometry analysis showed that the 5-6-FAM-labeled RNA/DNA nanostructure was more efficiently taken up by murine dendritic cell line, DC2.4 cells. In addition, the RNA/DNA nanostructure induced a high level of tumor necrosis factor-α release from DC2.4 cells. Immunostimulatory activity in vivo was evaluated by measuring cytokines/chemokines after injection to C57BL/6 mice. Intradermal injection of the RNA/DNA nanostructure resulted in an elevated interleukin-6 level at the injection site. These results indicate that the RNA/DNA nanostructure can be a useful adjuvant targeting human TLR8, because it has high structural stability, delivery efficiency and immune stimulation.
References	1. Kohta Mohri, Makiya Nishikawa, Natsuki Takahashi, Tomoki Shiomi, Nao Matsuoka, Kohei Ogawa, Masayuki Endo, Kumi Hidaka, Hiroshi Sugiyama, Yuki Takahashi, and Yoshinobu Takakura, *ACS Nano*, 6, 5931-5940, 2012 2. Shota Uno, Makiya Nishikawa, Kohta Mohri, Yuka Umeki, Noriyuki Matsuzaki, Yuki Takahashi, Haruyuki Fujita, Norimitsu Kadowaki, and Yoshinobu Takakura, *Nanomedicine*, 10, 765-774, 2014