Treatment of sensory nerve disorder by in vivo mRNA introduction using polyplex nanomicelle

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ABSTRACT SUMMARY
Messenger RNA (mRNA) is promising to provide proteins for therapeutic purposes. Using a novel non-viral system of polyplex nanomicelle possessing high stability and stealth property, BDNF-expressing mRNA was introduced into nose to treat olfactory disorder. By expressing BDNF extensively in submucous tissues containing endings of olfactory nerve, the mice showed significantly earlier recovery of smelling function with enhanced regeneration of neurons compared with control mice. This system achieved efficient protein production from mRNA in a safe and sustained manner, opening the door to new therapeutic strategies for nerve disorders.

INTRODUCTION
Nerve disorder is intractable due to poor capacity of nerve regeneration and the difficulties in approaching neural tissues. Various nerve growth factors have good indication for nerve protection and regeneration, however the administration of growth factors as a form of recombinant polypeptides has a problem of the transient and unstable effects, hampering its wide application for the treatment. In this study, we developed a novel non-viral system for in vivo mRNA introduction, and applied this system for the treatment of olfactory dysfunction by introduction of brain-derived neurotrophic factor (BDNF)-expressing mRNA.

EXPERIMENTAL METHODS
This in vivo mRNA delivery system is based on a self-assembly of mRNA and block copolymers composed of polyethylene glycol (PEG) and polyamino acids (Fig. 1 left). Polyplex nanomicelle possessing characteristic core–shell architecture is formed surrounded by PEG outer layer. The nanomicelle has a good potential to function as an effective mRNA-containing carrier with high stability and stealth property¹. Model mice possessing olfactory disfunction were created by i.p. injection of methimazole. The mRNA incorporated into nanomicelle was administrated by dropping into nose. The transgene expression by the mRNA was evaluated using Luciferase-expressing mRNA and acGFP-expressing mRNA. Then, for treatment of olfactory dysfunction, BDNF-expressing mRNA was introduced 5 days in a row, and the smelling function was analyzed by a behavioral test of seeking a hidden food. Finally, the intranasal mucous membrane structure was pathohistologically analyzed to evaluate the effect of BDNF-expressing mRNA for nerve protection and regeneration.

RESULTS AND DISCUSSION
The expression after luciferase-expressing mRNA introduction was well detected by
vivo Imaging System (IVIS) for more than two days (Fig. 1 right), whereas the administration of the mRNA as a form of naked mRNA, and the administration of luciferase-expressing plasmid DNA (pDNA) provided almost no expression. Histological analyses using acGFP-expressing mRNA revealed that the eGFP expression was obtained extensively in submucosus tissue (lamina propria) containing the endings of olfactory nerve (Fig. 2). The inflammatory responses after mRNA introduction using nanomicelle were significantly lower than those after naked mRNA introduction.

These results indicated that mRNA incorporated into nanomicelle was capable to express proteins in non-dividing cells in lamina propria. In addition, the sustained manner of protein expression for two days showed a clear advantage over recombinant protein, which would have very short length of effectiveness.

Then, BDNF-expressing mRNA was introduced using the nanomicelle 5 days in a row into mice showing olfactory disfunction. The mice receiving the mRNA showed significantly earlier recovery of smell function compared with controls receiving saline (Fig. 3). Pathohistological analyses with staining by a neuron-specific marker (Olfactory Marker Protein: OMP) revealed that in the mice receiving the mRNA, effective regeneration of OMP-positive neurons was achieved together with full recovery of the olfactory epithelium.

**CONCLUSION**

mRNA introduction is a promising approach to produce therapeutic proteins without any risk of insertion mutagenesis into the host genome. There are no needs of nuclear transport, allowing to provide protein expression in non-dividing cells. Thus, the in vivo mRNA introduction has feasibility for the treatment of nerve disorder.

In this study, we achieved the recovery of smelling function by introduction of BDNF-expressing mRNA using polyplex nanomicelle into nose. The safe and sustained manner of protein expression by in vivo mRNA introduction is opening the door to new therapeutic strategies for various nerve disorders.

**REFERENCES**


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