Intradermal RNAi therapeutic system by novel class of RNAi agents and functional peptide for atopic dermatitis

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ABSTRACT SUMMARY

In order to develop the non-invasive and effective intradermal RNAi therapeutic system for atopic dermatitis, the combination system of the novel class of RNAi agents (nkRNA, PnkRNA™), which is single-stranded RNAs, and stearoyl (STR)-peptide (STR-CysHisHisArgArgArgHisHisCys; STR-CH₃R₄H₂C) were developed. Two kinds of RNAi agents for mouse RelA, which is NF-κB family, with STR-CH₃R₄H₂C shows strong therapeutic effects in the atopic dermatitis-like ear of the disease model mice.

INTRODUCTION

Atopic dermatitis (AD) causes very low quality of life for patients, and effective treatment for AD is needed. siRNAs is expected the novel AD therapy because of the specifically gene silence effects for AD related factor. However, siRNAs could not exert the strong therapeutic effect due to the off-target effects and the low efficiency delivering into the target tissue and cells. We have reported that siRNAs for RelA, which is NF-κB family, with cell-penetrating peptide showed anti-allergy effects in AD-like mice ear skin.

A novel class of RNAi therapeutic agents (PnkRNA, nkRNA) has been already reported and evaluated their effectiveness. The production of the PnkRNA and nkRNA is simple, because PnkRNA and nkRNA are synthesized as single-stranded RNAs (ssRNAs) that spontaneously self-anneal, low-cost, large-scale production is possible. In addition, these novel RNAi agents have showed significant effectiveness in disease models and also superior resistance against nuclease degradation compared to canonical siRNAs. Furthermore, these RNAi-agents have not shown any immunotoxic by evaluating the induction of proinflammatory cytokines. Thus, PnkRNA and nkRNA are safe and might be employed in clinical applications instead of siRNAs.

STR-CH₃R₄H₂C was effective gene carrier peptide developed in our previous study. We previous demonstrated that STR-CH₃R₄H₂C strikingly enhanced in vitro siRNA silencing efficiency because of the stabilized formation and enhanced the cellular uptake by stearic acid (STR) and arginine (R), proton sponge effect by histidine (H), and ability to release siRNA from carrier in cytoplasm by cleavage of disulfide cross linkage of cysteine (C).

In the present study, to develop the novel and effective intradermal RNAi therapeutic system for AD, the combination system of the nkRNA, PnkRNA as the novel class of RNAi agents and STR-CH₃R₄H₂C were applied to AD-like ear in mice. We first determined the silencing effects of nkRNA, PnkRNA for mouse RelA, which is NF-κB family with STR-CH₃R₄H₂C in mouse macrophage or dendritic cells in vitro study and next evaluated the anti-allergy effects in the AD model mice following topical application of nkRNA or PnkRNA for mouse RelA with STR-CH₃R₄H₂C.

EXPERIMENTAL METHODS

The long RNA oligomers of nkRNA (62-mer) and PnkRNA (51-mer) were synthesized using solid phase as ssRNAs that self-anneal into a unique helical structure containing a central stem and two loops following synthesis. The PnkRNA were incorporated of the proline derivative molecule. CH₃R₄H₂C peptide (10-mer) was synthetized using the Fmoc strategy. The stearic acid was conjugated to N-terminal of CH₃R₄H₂C using solid-phase peptide synthesis method. STR-CH₃R₄H₂C and RNAi agents complexes (N/P: 10) were prepared by mixing peptides solution and RNAs solution for 30 min at room temperature.

JAWSII cells were seeded onto 24-well culture plates. After 48 h incubation, the cells were rinsed with PBS and then 900 µL of medium was added to each well. The complex solution (100 µL containing 50 nM of RNAi agents) was applied to each well. After 24 h, the medium was removed and replaced by 900 µL of 20% FBS, 100 µL of LPS solution (10 µg/mL) was added. After 8 h, the cells were washed with PBS and collected. The RelA mRNA was measured by RT-PCR. The relative RelA mRNA was calculated based on the RelA mRNA of control (LPS-stimulated cells) group.

AD-like ear skin lesions in NC/Nga mice were induced by repeated topical sensitization with 2,4-dinitrofluorobenzene. The non- or RelA-targeted
RNAi agents with STR-CH₂R₃H₂C were administrated AD-induced left ear two or three times a week for 2 weeks. Then, the ear thickness and clinical skin severity score were periodically examined throughout the study. Total clinical skin severity score was calculated from the sum of the five assessment categories (redness, hemorrhage, thickness, deformation and dryness).

RESULTS AND DISCUSSION
Fig. 1 shows the RelA mRNA expression in LPS-stimulated JAWS cells (5×10⁵/well) transfected with RNAi agents for mouse RelA/STR-CH₂R₃H₂C complex for 24h followed by LPS for 8h. As shown in Fig. 1, all of RNAs with STR-CH₂R₄H₂C showed significantly silencing effects compared with non-transfected cells (Control). Additionally, the silencing effects of target RelA mRNA by nkRNA and PnkRNA show a tendency to be higher than siRNA.

![Figure 1](image1.png)

Fig. 1 RelA mRNA expression in LPS-stimulated JAWS cells transfected with RNAs/STR-CH₂R₃H₂C complexes. RNAs were used siRelA, nkRNA, or PnkRNA for mouse RelA (50 nM) for 24h followed by LPS for 8h. The RelA mRNA was measured by RT-PCR. Mean ± S.D. (n=3). **P<0.01 (t-test)

The ear thickness and clinical skin severity score in AD-like mice after treated with non- or RelA-targeted RNAi agents (nkRNA, PnkRNA) with STR-CH₂R₃H₂C are shown in Fig. 2. From day 11, the ear thickness in untreated AD-like mice was largely increased. However, the ear thickness in RelA-targeted RNAi agents treated AD-like mice was not increased obviously. Additionally, the clinical skin severity score in untreated AD-like mice was increased significantly larger than that in RelA-targeted RNAi agents treated AD-like mice. In particular, the clinical skin severity score in RelA-targeted PnkRNA treated group was almost never increased. These results suggested that RelA-targeted two kinds of RNAi agents should be useful medicines for AD therapy, and the combination with STR-CH₂R₃H₂C was good system of AD therapy.

![Figure 2](image2.png)

Fig.2 The ear thickness and clinical skin severity score in AD-like mice after treated with non- or RelA-targeted novel RNAi agents with STR-CH₂R₃H₂C. NC/Nga mice were repeated sensitized with DNFB. The Untreated, (a) nkRNA or (b) PnkRNA (0.375 nmol) with STR-CH₂R₄H₂C in olive oil was treated on the AD-like left ear. Mean ± S.E. (n=4). **P<0.01 vs Untreated (t-test)

CONCLUSION
The novel class of RNAi agents (PnkRNA, nkRNA) with STR-CH₂R₃H₂C showed the highly gene silencing effects in mouse dendritic cells and strongly therapeutic effects against AD-like ear in mice. In conclusion, the combination of novel class of RNAi agents (PnkRNA, nkRNA) and STR-CH₂R₃H₂C was expected as a novel noninvasive topical therapeutic system for AD.

REFERENCES