RNAi-based modulation of IFN-γ signaling in skin

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1. 簡述論文的概要以及重大發現

IFN-γ signaling 不正常傳遞會引發自身免疫，導致各種不良反應，而阻斷 IFN-γ signaling 傳遞被認為是有效治療自身免疫的方法。

本研究利用 siRNA 能將目標 RNA 裂解(degradation) 的特性，將皮膚 Ifngr1 (IFN-γ receptor 1) 靜默(silencing)，以抑制 IFN-γ signaling 傳遞。由結果證實 siRNA 藥物擁有治療自身免疫疾病極大的潛力。

2. 對論文內容的提問

注入小鼠尾部的 siRNA 是否會影響其他器官或組織 IFN-γ signaling，導致其他部位的免疫反應受到影響，這是否需要考慮?

3. 論文的缺點與評論

此研究藉由體外和體內測試得到了正向的結果，證實 siRNA 作為治療自身免疫藥物的可行性，但是要如何預防抑制 Ifngr1 所帶來的不良影響仍需謹慎地看待防備。

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Qi Tang,[1,2] Jacquelyn Sousa,[1] Dimas Echeverria,[1] Xueli Fan,[2] Ying-Chao Hsueh,[2,4] Khashayar Afshari,[2] Nicholas MeHugh,[1] David A. Cooper,[1] Lorenc Vangjeli,[1] Kathryn Monopoli,[1,5] Ken Okamura,[2] Annabelle Biscans,[1] Adam Clauss,[3] John E. Harris,[2] and Anastasia Khvorova[1]

[1]RNA Therapeutics Institute, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA; [2]Department of Dermatology, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA; [3]LEO Pharma A/S, Industriparken 55, 2750 Ballerup, Denmark; [4]Immunology and Microbiology Program, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA; [5]Bioinformatics and Computational Biology Program, Worcester Polytechnic Institute, Worcester, MA 01609, USA

Aberrant activation of interferon (IFN)-γ signaling plays a key role in several autoimmune skin diseases, including lupus erythematosus, alopecia areata, vitiligo, and lichen planus. Here, we identify fully chemically modified small interfering RNAs (siRNAs) that silence the ligand binding chain of the IFN-γ receptor (*IFNGR1*), for the modulation of IFN-γ signaling. Conjugating these siRNAs to docosanoic acid (DCA) enables productive delivery to all major skin cell types local to the injection site, with a single dose of injection supporting effective IFNGR1 protein reduction for at least 1 month in mice. In an *ex vivo* model of IFN-γ signaling, DCA-siRNA efficiently inhibits the induction of IFN-γ-inducible chemokines, CXCL9 and CXCL10, in skin biopsies from the injection site. Our data demonstrate that DCA-siRNAs can be engineered for functional gene silencing in skin and establish a path toward siRNA treatment of autoimmune skin diseases.

INTRODUCTION

Interferon (IFN)-γ signaling promotes the progression of $CD8^+$ T cell-mediated autoimmune skin diseases, such as lupus erythematosus, alopecia areata, vitiligo, and lichen planus.[1–4] Autoreactive $CD8^+$ T cells in lesional skin produce IFN-γ that binds the IFN-γ receptor to activate the Janus kinase (JAK)-signal transducer and activator of transcription pathway, thereby stimulating the expression of IFN-γ-inducible chemokines, such as CXCL9, CXCL10, and CXCL11. These chemoattractants, in turn, promote the skin infiltration of autoreactive $CD8^+$ T cells and worsen autoimmunity (Figure 1A).[5–7]

The upregulation of IFN-γ and its related genes in skin biopsies from patients and mouse models of autoimmune skin diseases indicate that targeting the IFN-γ pathway may be an effective therapeutic strategy.[3,8,9] Indeed, the clinical off-label use of small molecule JAK inhibitors (e.g., ruxolitinib, tofacitinib, and baricitinib) has produced promising results in the treatment of alopecia areata and vitiligo.[10–13] These medications can be administered systemically or topically. Systemic treatments usually provide better outcomes for severe conditions when large areas of skin are involved, whereas topicals are good for patients with localized symptoms and incur fewer systemic side effects. Topical JAK inhibitors are efficacious in modulating IFN-γ-related autoimmunity; however, JAK inhibition could potentially affect other immune pathways and lead to undesirable activities.[14] Therefore, we hypothesize that locally targeting IFN-γ receptor, the upstream molecule, may be a viable approach to specifically modulate the IFN-γ signaling in skin.

Small interfering RNAs (siRNAs) are a novel class of drugs that harness endogenous RNA interference (RNAi) to enable specific and sustained modulation of gene expression.[15,16] Synthetic siRNAs offer several advantages over small molecule and antibody drugs, including ease of sequence-based design, which allows for rapid drug discovery, and the ability to target disease genes previously considered undruggable.[17] Currently, conjugate-mediated delivery of siRNAs is the dominant delivery platform in the clinic. The most clinically advanced siRNA conjugate, *N*-acetylgalactosamine (GalNAc), supports selective hepatocyte delivery through asialoglycoprotein receptor uptake and is the basis for multiple approved siRNA drugs.[18–20] GalNAc-conjugated siRNAs show an unprecedented duration of effect (>6 months after a single administration)[21] driven by compound entrapment in lysosomal and endosomal compartments, generating an intracellular depot of the drug with slow release.[22] While the usefulness of GalNAc is limited to the liver, hydrophobic conjugates support delivery to extra-hepatic organs, including skin.[23,24] Among hydrophobic conjugates, docosanoic acid (DCA) shows promise for safe, multicellular siRNA delivery both locally and systemically.[23,25]

Here we report the rational development of therapeutic siRNAs that silence the ligand binding chain of the IFN-γ receptor (*IFNGR1*) for the modulation of IFN-γ signaling. We screened a panel of fully chemically modified siRNAs *in vitro* and identified multiple

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Correspondence: John E. Harris, Department of Dermatology, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA.
E-mail: john.harris@umassmed.edu
Correspondence: Anastasia Khvorova, RNA Therapeutics Institute, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA.
E-mail: anastasia.khvorova@umassmed.edu