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COMMENTARY

The potential of RNA therapeutics in dermatology

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ABSTRACT

Ribonucleic acid (RNA) therapeutics hold great potential for the advancement of dermatological treatments due to, among other reasons, the possibility of treating previously undruggable targets, high specificity with minimal side effects, and ability to include multiple RNA targets in a single product. Although there have been research relating to RNA therapeutics for decades, there have not been many products translated for clinical use until recently. This may be because of challenges to the application of RNA therapeutics, including the dearth of effective modes of delivery to the target, and rapid degradation of RNA in the human body and environment. This article aims to provide insight on (1) the wide-ranging possibilities of RNA therapeutics in the field of dermatology as well as (2) how key challenges can be addressed, so as to encourage the development of novel dermatological treatments. We also share our experience on how RNA therapeutics have been applied in the management of hypertrophic and keloid scars.

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The potential of RNA therapeutics

Ribonucleic acid (RNA) therapeutics involving, among others, microRNAs (miRNAs), small interfering RNAs (siRNAs) and/or antisense oligonucleotides (ASOs) hold great potential for the advancement of medical treatments in dermatology.

First, there are now novel ways to treat several dermatological conditions where existing treatments have been largely unsatisfactory. Critically, only around 1.5% of the human genome is translated into proteins;[1,2] of 20,300 protein-coding genes, only approximately slightly less than 4500 genes are considered druggable,[3] as not all proteins contain active sites for small molecule binding.[1,4] RNA therapeutics are thus able to provide sequence-specific gene therapy to many previously undruggable targets.[5] ASOs, siRNAs and miRNAs can overcome the major limitation of traditional drug molecules that can only target certain protein classes—these 3 do so by downregulating the expression of mRNA transcripts, which is useful because many diseases result from the expression of undesired or mutated genes, or from overexpression of certain normal genes.[6] Separately, RNA therapeutics can also include messenger RNA delivery to induce expression of proteins of interest. For example, the treatment of metastatic melanoma, a life-threatening form of skin cancer purported to be resistant to radiotherapy and chemotherapy, has demonstrated encouraging results,[7] with both translational repressing and mRNA-inducing forms of RNA therapeutics.[8]

Second, the high specificity of RNA therapeutics can potentially reduce the likelihood of side effects commonly associated with small molecules. This is due to, among other reasons, the ability to design an RNA sequence that is effective and specific to the target sequence to minimise any off-target effect.[6] Furthermore, application of local siRNA therapy can be limited to the area of affected skin, thereby minimalizing systemic toxicity.[9]

Third, the ability of certain RNA therapeutics to include multiple targets in a single product is another advantage. As miRNAs can inhibit numerous target genes,[10] miRNA therapeutics can be applied in the treatment of complex multigenic diseases, such as cancers and neurodegenerative disorders. [6] While siRNAs appear to be limited as they target only 1 specific gene (unlike miRNAs that can impact multiple genes), this can be overcome by employing multiple siRNA sequences in a single formulation.[6]

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Other positive attributes of RNA therapeutics include the relative cost-effectiveness,[1,7] ease in development,[1,7] as well as ability to upscale manufacturing efficiently in comparison to small molecules and biologics,[1] which result in a corresponding further reduction in cost.

Key challenges and potential solutions

Challenges to application of RNA therapeutics

Despite the existence of research relating to RNA therapeutics since decades ago,[9] as well as its potential, there have not been many products translated for clinical application until recently. This may be because of challenges to the application of RNA therapeutics. Some major hurdles are:

(1) delivery: the dearth of effective modes of delivery to the target tissue and cell type;[1,5,6,10]

(2) RNA instability: RNA degrades rapidly in the human body and environment due to the ubiquitous presence of nucleases; hence naked RNA structures that are not offered any protection (e.g. by delivery vehicles) are unstable;[1,5,6,10]

(3) side effects: undesirable consequences such as off-target effects;[6,10] and

(4) possible immunogenicity: tolerability concerns due to the immune-stimulatory potential of synthetic RNA therapeutics.[10]

Overcoming hurdles to delivery and RNA instability

The skin is an easily accessible organ for therapeutic delivery. Large molecule-size RNAs can be delivered through intact skin via transdermal delivery methods such as dissolvable hyaluronic acid (HA) microneedles. For skin diseases with compromised skin barrier, such as eczema and wounds, RNA can be delivered more easily into the dermis, without additional need for transdermal delivery modalities.

Delivering RNA therapeutics past the skin barrier is an important step to achieving therapeutic efficacy. A number of delivery modalities explored in the context of dermatology include (1) physical methods, such as: microneedles, intradermal injection, tape-stripping, ballistic methods/gene gun, cavitational ultrasound/sonophoresis, electroporation, jet injection, and iontophoresis; and (2) chemical means such as chemical enhancers and lipid based systems.[9] To provide a brief overview, some delivery techniques that have been studied for certain dermatological conditions are outlined in Supplementary Table S1. Further research may be required to identify the most suitable technology for different applications,[9] and the illustrations are non-exhaustive.

Simultaneously, RNA instability can be addressed using chemical modifications to improve RNA stability, while encapsulation of RNAs using nanoparticles can also reduce exposure to environmental nucleases.

Mitigation of off-target effects

Off-target effects occur when partial mismatch of siRNA and off-target genes result in binding and subsequent translational repression. Strategies to reduce off-target effects depend on the underlying RNA therapeutic. These include (1) applying the lowest possible RNA therapeutic dosing, as the impact of side effects are largely dependent on concentration levels;[6,10] (2) utilising chemical modifications;[6,10] and (3) carefully designing an RNA sequence based on its unique profile, so as to maximise the specificity and minimise any off-target effect.[6] As some RNAs are similarly expressed in multiple cell types, uptake of RNA therapeutic by non-target cells can contribute to undesired non-specificity. This can be addressed by conjugating ligands that bind to target cell-specific receptors to improve uptake. In respect of dermatological treatments for conditions that affect parts of the skin, side effects can be further mitigated by localising RNA therapy to the area(s) of affected skin, thus minimalizing potential side effects.[9]

Minimise the likelihood of possible immunogenicity

The body's immune system recognises foreign RNA structures by pathogen-associated molecular pattern receptors such as toll-like receptors (TLRs). Although there is a possibility for tolerability issues to arise, leading to adverse immune effects,[10] there are ways to prevent such negative outcomes. A main method would be to carefully design the RNA sequence,[6] and conduct screening to select RNA therapeutics with the smallest potential immunogenicity.[10] Other approaches include (1) chemical modifications, (2) using shorter RNA sequences (as it is purported that efficient activation of TLRs requires a length of at least 21 nucleotides for single-stranded RNA) and/or (3) adjusting the treatment regimen to reduce the dose (e.g. by using combination therapies like RNA interference with additional therapeutic regimes).[10]

Our experience in applying RNA therapeutics in the management of pathological scars

We believe that RNA therapeutics can be efficacious as dermatological treatments, and we have applied them in the management of hypertrophic

and keloid scars at the National Skin Centre, Singapore.

Wound fibrosis is a multifactorial process, and one of the main mechanisms driving hypertrophic scar formation is excessive collagen deposition, which is linked to expression of the matricellular collagen-binding protein secreted protein acidic and cysteine-rich (SPARC).[5] Dissolvable and biocompatible HA microneedle patches loaded with siRNA for SPARC (siSPARC) to silence the SPARC protein were used.[5] To improve siRNA stability and cellular uptake, tyramine-modified gelatin (Gtn-Tyr) nanoplexes were used to form a siSPARC/Gtn-Tyr nanoplex, which effectively reduces collagen production, thereby potentially preventing excessive scar formation.[5]

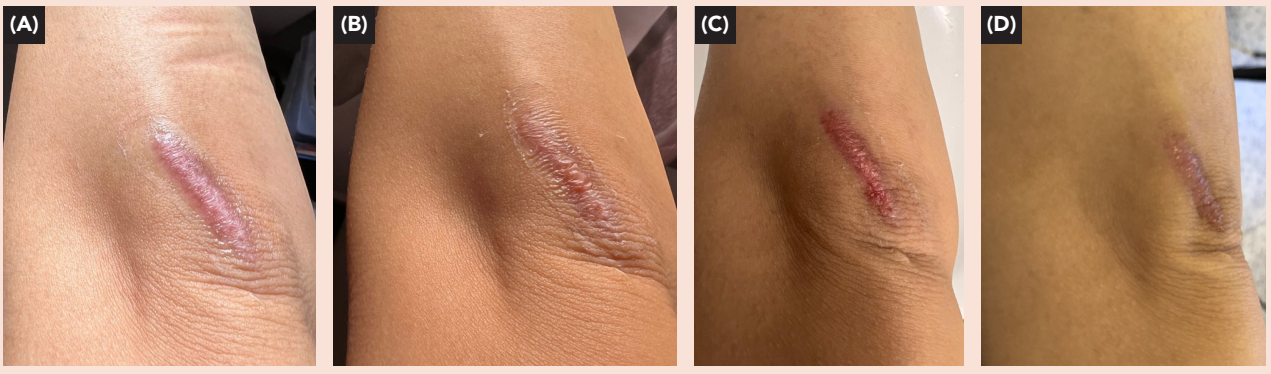
One illustration is an individual who traumatised her right elbow 6 weeks earlier and developed a hypertrophic scar with symptoms of pain and allodynia shown in Fig. 1 (pain score of 8/10). After 2 days of daily application of the siSPARC/Gtn-Tyr nanoplex-loaded HA microneedle patch, the patient reported resolution of pain, with significant improvement in allodynia, scar erythema and thickness. Five days post daily application, allodynia had fully subsided, and the scar became flatter and more pliable. After 22 days of daily application, the scar had visibly flattened with sustained improvement in hypersensitivity and erythema. When the patient self-discontinued the application for 2 days, the allodynia recurred (pain score 2/10). However, this abated with re-application of the microneedle patch. When the patient ceased application of the microneedle patch, the hypertrophic scar gradually increased in size, but did not reach the baseline scar volume. The siSPARC/Gtn-Tyr nanoplex-loaded HA microneedle patch is a promising transdermal RNA therapeutic for topical delivery of siRNA across the skin barrier to treat and prevent hypertrophic scars.[5]

This treatment modality is particularly suitable for (1) patients who prefer non-steroidal treatment due to concerns of adverse effects of steroids, including skin atrophy and telangiectasia; (2) patients who suffer from steroid atrophy; (3) patients with sensitive scars or who are otherwise unable to tolerate intralesional steroid injections, e.g. children; and (4) prevention of scar recurrence after improvement with intralesional steroid injections.

For keloid scars, we primarily use the siRNA microneedle patches to prevent the recurrence of keloids after treatment, which is a major issue in the treatment of keloids. Intralesional injection of triamcinolone is the most commonly used treatment worldwide and recurrence rates range from 33–50%.[11] We typically offer the siRNA microneedle patches to patients who experience recurrence after treatment, starting once the primary treatment flattens the keloids. Intralesional steroid injection is not suitable for such a preventive measure, as long-term steroid usage often lead to cutaneous side effects and patients have to regularly visit the dermatology clinic on a long-term basis to receive the injections.

Dermatology is a field with good potential for the implementation of RNA therapeutics. The positive observations thus far are proof of concept of the utility and potential of RNA therapeutics. They portend clinically-effective treatments for various dermatological conditions to be developed in the near future.

Fig. 1. Application in a case of hypertrophic scar. (A) At baseline, there was pain and allodynia with a pain score 8/10. (B) Day 2 of daily application of siRNA-embedded HA dissolvable microneedle patch: complete resolution of pain, with significant improvement in allodynia, scar erythema and thickness. (C) Day 5: the scar was flatter and softer, with a total resolution of allodynia. (D) Day 22: there was a sustained improvement of allodynia and significant improvement of the scar erythema and thickness compared to baseline. Subsequently, when application of the patches ceased for 2 days, the allodynia recurred, with a pain score 2/10. (All photographs are used with permission and consent is required for reproduction).



Disclosure

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REFERENCES

1. Damase TR, Sukhovershin R, Boada C, et al. The Limitless Future of RNA Therapeutics. Front Bioeng Biotechnol 2021;9:628137.
2. Ezkurdia I, Juan D, Rodriguez JM, et al. Multiple evidence strands suggest that there may be as few as 19 000 human protein-coding genes. Hum Mol Genet 2014;23:5866-78.
3. Finan C, Gaulton A, Kruger FA, et al. The druggable genome and support for target identification and validation in drug development. Sci Transl Med 2017;9:eaag1166.
4. Hopkins AL, Groom CR. The druggable genome. Nat Rev Drug Discov 2002;1:727-30.
5. Chun YY, Tan WWR, Vos MIG, et al. Scar prevention through topical delivery of gelatin-tyramine-siSPARC nanoplex loaded in dissolvable hyaluronic acid microneedle patch across skin barrier. Biomater Sc 2022;10:3963-71.
6. Lam JK, Chow MY, Zhang Y, et al. siRNA versus miRNA as Therapeutics for Gene Silencing. Mol Ther Nucleic Acids 2015;4:e252.
7. Kieser RE, Khan S, Bejar N, et al. The Dawning of a New Enterprise: RNA Therapeutics for the Skin. J Dermatol & Skin Sci 2023;5:4-13.
8. Hotz C, Wagenaar TR, Gieseke F, et al. Local delivery of mRNA-encoded cytokines promotes antitumor immunity and tumor eradication across multiple preclinical tumor models. Sci Transl Med 2021;13:eabc7804.
9. Geusens B, Sanders N, Prow T, et al. Cutaneous short-interfering RNA therapy. Expert Opin Drug Deliv 2009; 6:1333-49.
10. Winkle M, El-Daly SM, Fabbri M, et al. Noncoding RNA therapeutics—challenges and potential solutions. Nat Rev Drug Discov 2021;20:629-51.
11. Morelli Coppola M, Salzillo R, Segreto F, et al. Triamcinolone acetonide intralesional injection for the treatment of keloid scars: patient selection and perspectives. Clin Cosmet Investig Dermatol 2018;24;11:387-96.