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Quantum molecular resonance ameliorates atopic dermatitis through suppression of IL36G and SPRR2B

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Atopic dermatitis (AD) is a chronic, pruritic skin disease characterized by inflammation and skin lesion cornification. While the use of corticosteroids like dexamethasone (DXM), an anti-inflammatory drug, improves symptoms temporarily and quickly, this use is not a cure. Thus, we aimed to identify a new therapeutic strategy for AD using quantum molecular resonance (QMR), a novel non-invasive technique with an electromagnetic field-based therapeutic approach as an alternative to pain killers. An AD mouse model presenting AD-like skin lesions was generated by treating BALB/c mice with dinitrochlorobenzene (DNCB), and then DNCB-induced AD mice were administered DXM or QMR, and the change of AD-like skin lesions was observed. QMR ameliorated AD-like skin lesions in DNCB-induced AD mice and reduced the numbers of infiltrated mast cells and macrophages in mouse skin. QMR also alleviated thickening of the epidermis and restored integrity of the epidermal basement membrane. Several genes regulated by DNCB and counter-regulated by QMR were identified through transcriptome analysis in mouse skin, and RNA silencing experiments on these genes in TNF-α/IFN-γ- or DNCB-treated human keratinocytes revealed that IL36G and SPRR2B play important roles in inflammation and keratinization. The expression of IL36G and SPRR2B was significantly reduced by QMR in skin of DNCB-induced AD mice. These results underscore the promising role of QMR in ameliorating AD characterized by inflammation and skin lesion hyperkeratosis via targeting IL36G and SPRR2B.

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INTRODUCTION

Atopic dermatitis (AD) is a common, chronic inflammatory skin condition characterized by dry, itchy, and eczematous lesions that lead to redness and inflamed patches on the skin. AD can result in recurring episodes, significantly affecting the quality of life, and causing emotional distress (1). AD is a complex disease with multiple potential triggers, including genetic factors, immune system dysregulation, skin barrier dysfunction, and environmental factors like allergens, changes in humidity, and dry air. One prominent feature of AD is mast cell degranulation, which plays a major role in allergic reactions. When mast cells or basophils are activated by various stimuli, such as the binding of allergens to immunoglobulin E (IgE) antibodies, mechanical irritation, or stress, these cells release their granule contents, including cytokines, chemokines, and inflammatory factors like histamine, into the surrounding tissue (2).

Accumulated evidence also suggests a connection between AD and allergic inflammation, hyperproliferation of keratinocytes, and disruptions in the skin barrier. The hyperproliferation of keratinocytes is a common characteristic of various benign skin disorders, including chronic inflammatory conditions like psoriasis and AD (3). Specific genetic defects within keratinocytes can lead to disorders in the cornification process of the skin, which can exhibit both local and systemic atopic features (4). The precise cause of AD is unknown and not yet defined. Current treatment approaches primarily rely on therapeutic painkillers, including topical and systemic corticosteroids like dexamethasone (DXM), anti-inflammatory drugs, phototherapy, calcineurin inhibitors, and immunosuppressive agents (5). While showing promise in managing AD, prolonged exposure to these drugs can result in various irreversible side effects and addiction (6).

Quantum molecular resonance (QMR) is a novel non-invasive technique with an electromagnetic field-based therapeutic approach as an alternative to pain killers. QMR generates distinct wave patterns at high frequencies, ranging from 4 to 64 MHz, using low-intensity electric fields (7). Numerous investigations have explored the potential impacts of low-level electromagnetic fields on diverse biological processes, including wound healing, reduction of inflammation, angiogenesis, and differentiation

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(8-10). Recent studies demonstrate that QMR exhibits therapeutic benefits for various clinical conditions such as dry eye disease, osteoarthritis-related inflammation, hard-to-heal extremity wound, postoperative perilesional edema, and ecchymosis in patients with rhino septoplasty (11-15). However, the efficacy of QMR in AD is unknown. In this study, we generated a dinitrochlorobenzene (DNCB)-induced AD-like mouse model to test for mitigating effects of QMR on skin lesions in AD. We aimed to identify the potential of QMR as a therapy for AD, characterized by inflammation and hyperkeratosis in the skin, and the underlying mechanisms for QMR's effects.

RESULTS

QMR ameliorates skin lesions in the DNCB-induced AD mouse model

To investigate the potential benefits of QMR in treating AD, we generated an AD mouse model presenting AD-like skin lesions by applying DNCB to the dorsal skin of BALB/c mice (DNCB group). The mice in the DNCB group displayed AD-like symptoms, including infiltrative erythema, edema, pruritus, hemorrhage, erosion, scratching, excoriation, and dryness, which are characteristic clinical manifestations of AD (16). Prior to QMR treatment in the AD mouse model, we applied QMR at pulse powers of 5, 10, and 20 W to the dorsal skin of normal BALB/c mice to determine the optimal pulse power for QMR treatment (Supplementary Fig. 1A, B). While QMR application at pulse powers of 5, 10, and 20 W did not cause any changes in body weight and dorsal skin surface in normal BALB/c mice (Supplementary Fig. 1C, D), various immunohistochemistry including PAS staining and F4/80 staining showed that a pulse power of 20 W caused some abnormalities in epidermal thickness and immune cell infiltration (Supplementary Fig. 2). After determining the optimal QMR pulse power, DNCB-induced AD mice were treated with 3 mg/kg of DXM (DNCB + DXM group) or 10 W pulse of QMR (DNCB + QMR group) (Fig. 1A). To assess the therapeutic effects of QMR, we evaluated the severity of AD in skin lesions. Gross images of the dorsal skin exhibited severe erythema, edema, and eczematous skin lesions in the DNCB group, while markedly alleviated AD symptoms were observed in both the DNCB + DXM group and DNCB + QMR groups (Fig. 1B). No significant change in body weight was observed in mice in any experimental group during the period of treatment, suggesting that QMR does not cause the adverse effects of weight loss (Fig. 1C). When we scored the severity of AD symptoms including erythema/hemorrhage, edema, scarring/dryness, and excoriation/erosion, SCORAD indices of both the DNCB + DXM and DNCB + QMR groups were significantly decreased in comparison with the DNCB group, indicating a therapeutic effect of QMR against AD skin lesions (Fig. 1D).

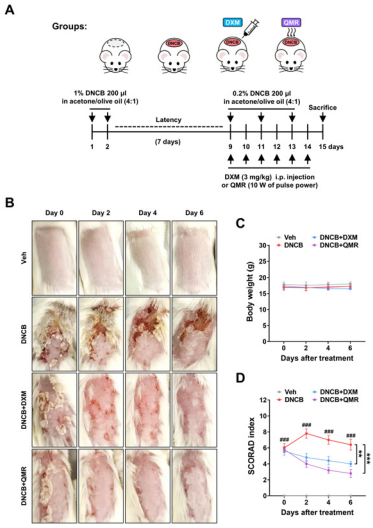

Fig. 1. QMR ameliorates skin lesions in DNCB-induced AD mice. (A) Schematic diagram of the generation of a dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD) mouse model and the experimental design. BALB/c mice were divided into four groups, five mice per group: the Veh group (vehicle-treated mice), the DNCB group (DNCB-treated mice), the DNCB + DXM group (3 mg/kg of dexamethasone (DXM) intraperitoneal injection-treated DNCB mice), and the DNCB + QMR group (10 W pulse of quantum molecular resonance (QMR)-treated DNCB mice). (B) Representative images of mouse skin lesions in each experimental group during QMR treatment. (C) Body weight was measured every other day during QMR treatment (n = 5 per group). (D) Scoring AD (SCORAD) index was evaluated as described in METHODS during QMR treatment (n = 5 per group). The severity of dermatitis was measured based on symptoms of erythema/hemorrhage, edema, scarring/dryness, and excoriation/erosion. All data are shown as mean ± SD. **P < 0.01, $^{\#\#\#}$P or ***P < 0.001 by two-way ANOVA with Bonferroni's test. ($^{\#}$comparison between Veh group and DNCB group; *comparison between DNCB group and DNCB + QMR group in (D)).

QMR suppresses skin inflammation by reducing the infiltration of mast cells and macrophages in the DNCB-induced AD mouse model

The release of inflammatory substances by immune cells in AD skin lesions is a major contributor to the characteristic features of the condition. Especially, the inflammatory substances released by mast cells, including IgE, histamine, cytokines, and other mediators, contribute to skin inflammation, persistent itching, and the development of a rash (17). Thus, we investigated whether QMR reduces mast cell infiltration in skin lesions of DNCB-induced AD mice. TB staining in mouse skin sections revealed that infiltration of TB^{+} mast cells was significantly reduced in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group, accompanied by a significant reduction in serum IgE levels (Fig. 2A and Supplementary Fig. 3). Similarly, the accumulation of macrophages is a striking hallmark of the inflammatory process in AD, and these macrophages infiltrate the skin and perpetuate inflammation (18). Therefore, we investigated whether QMR decreases

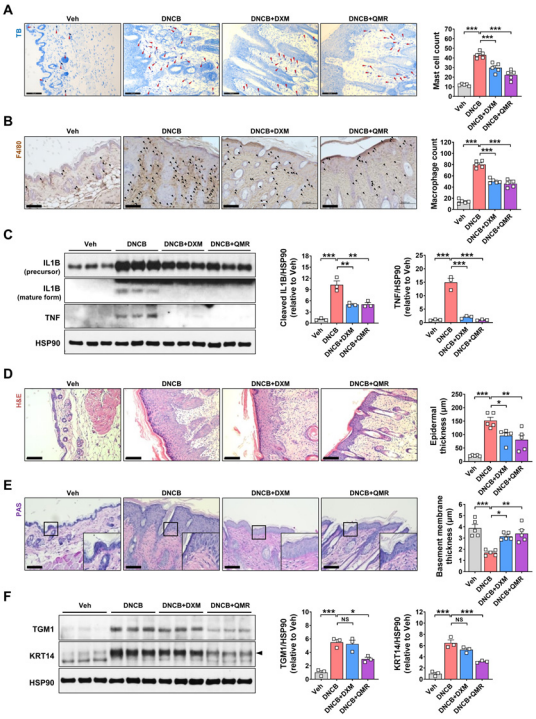

Fig. 2. QMR alleviates skin inflammation and hyperkeratosis in DNCB-induced AD mice. (A) Toluidine blue (TB) staining in mouse skin tissue sections. Representative images are presented (left), and red arrow heads indicate TB^+ mast cells (Scale bar, 100 μm). The number of infiltrated mast cells was counted (n = 5 per group) (right). (B) Immunohistochemical staining of F4/80 in mouse skin tissue sections. Representative images are presented (left), and black arrow heads indicate $F4/80^+$ macrophages (Scale bar, 100 μm). The number of infiltrated macrophages was counted (n = 5 per group) (right). (C) Immunoblot analysis of an extract of mouse skin tissue using antibodies against IL1B or TNF (left). Densitometric value of cleaved IL1B and TNF bands normalized to HSP90 bands (right). (D) Hematoxylin and eosin (H&E) staining of mouse skin tissue sections. Representative images are presented (Scale bar, 100 μm) (left). Thickness of the epidermis was measured (n = 5 per group) (right). (E) Periodic acid-Schiff (PAS) staining in mouse skin tissue sections. Representative images are presented (left), and inset images were magnified (Scale bar, 100 μm). Thickness of epidermal basement membrane was evaluated (n = 5 per group) (right). (F) Immunoblot analysis of extract of mouse skin tissue using antibodies against TGM1 or KRT14 (left). Black arrowhead indicates the true band of KRT14. Densitometric value of TGM1 and KRT14 bands normalized to HSP90 bands (right). All data are shown as mean ± SD. *P < 0.05, **P < 0.01 and ***P < 0.001 by one-way ANOVA with Tukey's test. NS, not significant.

the accumulation of macrophages in skin lesions of DNCB-induced AD mice. Immunohistochemical staining of F4/80 in mouse skin sections revealed that the number of $F4/80^+$ macrophages was notably reduced in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group (Fig. 2B). The gene expression level of *Adgre1*, a macrophage marker, was also reduced in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group (Supplementary Fig. 4A). Since the signaling pathways associated with inflammatory cytokines such as interleukin-1 beta (IL1B), interleukin-6 (IL6), and tumor necrosis factor alpha (TNF) amplify the inflammatory response in AD (19), we investigated QMR regulation of expression of genes associated with these inflammatory cytokines. Relative mRNA levels of genes including *Il1b*, *Il6*, and *Tnf* were markedly decreased in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group (Supplementary Fig. 4B). Immunoblot analysis also revealed decreased expression of precursor IL1B and TNF in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group (Fig. 2C). Furthermore, cleaved IL1B, an active form of IL1B, was significantly reduced in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group (Fig. 2C). In addition, relative mRNA levels of genes of AD-related key cytokines including *Il4, Il13*, and *Il31* were notably decreased in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group, suggesting that QMR contributes to the treatment of AD by reducing the gene expression of inflammatory cytokines related to AD (Supplementary Fig. 4C). These results suggest that QMR reduces the infiltration of immune cells that cause inflammation and decreases the secretion of inflammatory cytokines in AD skin lesions.

QMR alleviates hyperkeratosis and thickening of epidermis in the DNCB-induced AD mouse model

Epidermal keratinocytes are the primary cells in human skin, responsible for the skin's barrier and structural support. Hyperkeratosis is the excessive thickening of the outer layer of the skin (epidermis), a common feature of AD. This thickening is often due to overproduction of skin cells, leading to dry, scaly, and itchy skin. The accumulation of excess skin cells can disrupt the skin's natural barrier, making the skin more vulnerable to allergens and irritants (20). In AD, defects or alterations in the basement membrane may be present. These can lead to a compromised skin barrier, allowing allergens, irritants, and microbes to penetrate more easily, increasing inflammation and immune responses in the skin (21). To investigate the effects of QMR on skin thickening and tissue membrane integrity under AD conditions, histological analysis was conducted. H&E staining of mouse skin sections showed significant rescue of epidermal thickness in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group (Fig. 2D). PAS staining of mouse skin sections displayed notable restoration of ruptured basement membranes in both the DNCB + DXM and DNCB + QMR groups in comparison with the DNCB group (Fig. 2E). Furthermore, immunohistochemical staining showed that the relative optical density (O.D.) of DAB^+ proteins related with skin barrier formation, such as filaggrin (FLG) and loricrin (LOR), was significantly reduced in the DNCB group compared to the control group (Supplementary Fig. 5A). However, there was a trend toward recovery in the DNCB +

QMR group compared to the DNCB group (Supplementary Fig. 5A). Similarly, immunoblot analysis indicated significantly decreased FLG and LOR expression in the DNCB group, with a tendency for recovery in the DNCB + QMR group (Supplementary Fig. 5B). Of importance, relative mRNA levels of genes associated with keratinization (transglutaminase 1 (*Tgm1*) and keratin 14 (*Krt14*)) were significantly decreased only in the DNCB + QMR group compared to the DNCB group (Supplementary Fig. 4D). Consistently, immunoblot analysis also showed that the expression of TGM1 and KRT14 was decreased only in the DNCB + QMR group compared to the DNCB group, suggesting that QMR, but not DXM, regulates keratinization in skin directly (Fig. 2F). Collectively, these results implicate that QMR alleviates hyperkeratosis, reduces epidermal thickening, and improves the integrity of tissue membranes in AD skin lesions.

RNA sequencing data analysis reveals differential transcriptome profile by QMR stimulation in skin of the DNCB-induced AD mouse model

To explore the potential function of QMR and its molecular mechanism in the skin of AD mice, we performed an RNA sequencing data analysis of mouse skin of all experimental groups. We search up- or down-regulated genes (fold change $>$ 2.0, P value $<$ 0.05) in the DNCB group and reversely regulated genes (fold change $>$ 2.0, P value $<$ 0.05) in the DNCB + QMR group. Heatmap and volcano plot analyses were performed to assess the pattern of differentially expressed genes (DEGs) (Fig. 3A, B). To elucidate the molecular and cell biological information of these genes, we conducted Gene Ontology (GO) and KEGG pathway analyses. GO analysis revealed that QMR treatment influenced the expression of genes associated with biological processes such as response to stimuli, cell differentiation, protein metabolic processes, signaling, immune system processes, and enzyme regulation (Fig. 3C). KEGG pathway analysis demonstrated that QMR treatment affected pathways related to immune response, keratinization, keratinocyte differentiation, epidermis development and epithelial cell differentiation (Fig. 3D). Based on transcriptome analysis, we identified 24 genes that were opposingly regulated by DNCB and QMR treatments. Among these, 13 genes associated with immune response and skin keratinization showed significant changes. From this subset, seven genes with human homologues expressed in skin were selected as final candidate genes. These seven genes—*Serpinb12*, *Il36g*, *Ereg*, *Ctsc*, *Rptn*, *Sprr2b*, and *Ctsa1*—were found to be regulated by QMR treatment in skin lesions of AD mouse model and are highlighted in Fig. 3B.

Inhibition of IL36G and SPRR2B leads to suppression of inflammation and keratinization in TNF-α/IFN-γ-treated or DNCB-treated human keratinocytes

To identify regulators having QMR-mediated mitigating effects against AD, we generated an in vitro model mimicking the DNCB-induced mouse model. We treated human keratinocytes, HaCaT cells, with a combination of TNF-α and interferon gamma

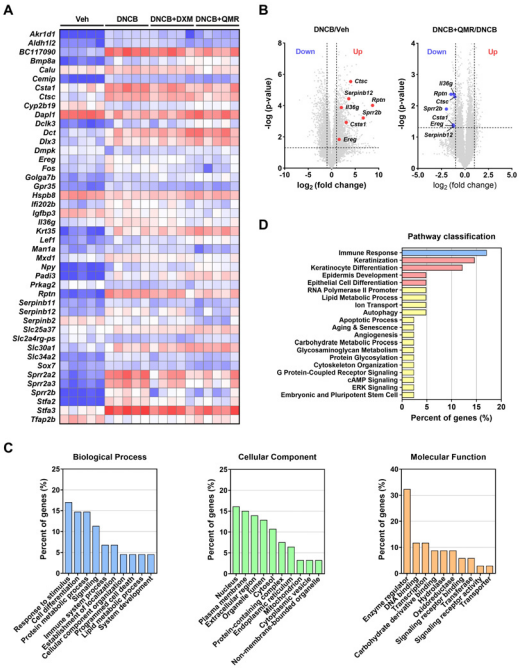

Fig. 3. RNA-sequencing data analysis reveals a differential transcriptome profile by QMR stimulation in DNCB-induced AD mice. (A) Heatmap showing differentially expressed genes (DEGs) from an RNA-sequencing data analysis using mouse skin tissue of each experimental group (n = 5 per group). (B) Volcano plot showing significantly up- or down-regulated genes by DNCB (left) and reversely regulated genes by QMR treatment (right). Data points for seven candidate genes are presented (red, up-regulated; blue, down-regulated). (C) The 10 most highly enriched Gene Ontology (GO) terms for categories of biological process, cellular component, and molecular function. (D) Pathway classification analysis showing the five most highly involved pathways associated with DEGs between significantly regulated genes by DNCB and reversely regulated genes by QMR treatment.

(IFN-γ) (22), and then incubated these TNF-α/IFN-γ-treated HaCaT cells with siRNAs against the seven candidate genes discovered by transcriptome analysis. Relative mRNA levels of genes associated with inflammation (*IL1B*, *TNF*, and *IL6*) and keratinization (*TGM1* and *KRT14*) were significantly reduced by siRNA against *IL36G* and *SPRR2B*, respectively (Supplementary Fig. 6A, B). Also, the relative mRNA levels of genes related to stem cell-based regeneration (nestin (*NES*), *CD34*, and *CD44*) were significantly increased by siRNAs against *IL36G* and *SPRR2B*, suggesting that inhibition of *IL36G* and *SPRR2B* shows a gene signature indicative of skin regeneration (Supplementary Fig. 6C). Consistently, immunoblot analysis demonstrated that knockdown of *IL36G* and *SPRR2B* suppresses the expression of inflammatory cytokines and keratinization-related proteins in TNF-α/IFN-γ-treated or DNCB-treated HaCaT cells (Supplementary Fig. 7A-D). Meanwhile, immunoblot analysis revealed that the expression of skin barrier-related proteins

was reduced in TNF-α/IFN-γ-treated or DNCB-treated HaCaT cells, with a tendency for recovery upon knockdown of *SPRR2B* (Supplementary Fig. 8A, C). The relative mRNA levels of genes associated with skin barrier formation also showed reduced expression in these treated cells, with a tendency for recovery following *SPRR2B* knockdown (Supplementary Fig. 8B, D). The knockdown of *IL36G* and *SPRR2B* by each siRNA was validated by real-time RT-PCR and immunoblot analysis (Supplementary Fig. 9A, B). While DXM effectively lowered the expression of genes associated with inflammation, it did not affect those linked to keratinization (Supplementary Fig. 6, 7). These results suggest that inhibition of IL36G and SPRR2B alleviates the AD-like phenotype in TNF-α/IFN-γ-treated or DNCB-treated human keratinocytes.

QMR inhibits the expression of IL36G and SPRR2B in skin lesions of DNCB-induced AD mice

A recent study reported that IL36G, a key initiator of inflammation, is expressed by keratinocytes and is increased in skin and sera of patients with AD (23). Other research has shown an altered pattern in small proline-rich (SPRR) protein expression in atopic eczema skin lesions and in an asthma model (24). Thus, we tested for QMR alleviation of the AD-like phenotype through suppression of IL36G and SPRR2B in DNCB-induced AD mice. Immunohistochemical staining in mouse skin sections revealed that the number of IL36G$^+$ cells was notably reduced in both the DNCB + DXM and DNCB + QMR groups compared to the DNCB group and accompanied significantly reduced expression of the *Il36g* gene (Fig. 4A, B). Immunohistochemical staining in mouse skin sections also demonstrated that the number of SPRR2B$^+$ cells were markedly decreased only in the DNCB + QMR group compared to the DNCB group, accompanied by significantly reduced expression of the *Sprr2b* gene (Fig. 4C, D). Consistently, immunoblot analysis also demonstrated significantly decreased expression of IL36G and SPRR2B in the DNCB + DXM group compared to the DNCB group (Fig. 4E). These results suggest that QMR ameliorates the AD-like phenotype through regulation of IL36G and SPRR2B in skin lesions of DNCB-induced AD mice.

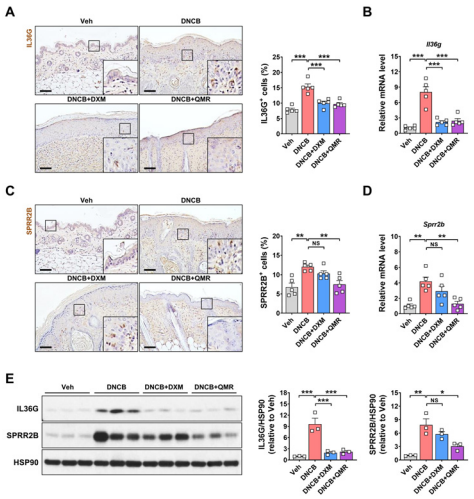

Fig. 4. QMR inhibits the expression of IL36G and SPRR2B in skin lesions of DNCB-induced AD mice. (A) Immunohistochemical staining of IL36G in mouse skin tissue sections. Representative images are presented (left), and inset images were magnified (Scale bar, 100 μm). Percentage of IL36G$^+$ cells among total cells was estimated (n = 5 per group) (right). (B) Relative gene expression level of *Il36g* in mouse skin tissue (n = 5 per group). (C) Immunohistochemical staining of SPRR2B in mouse skin tissue sections. Representative images are presented (left), and inset images were magnified (Scale bar, 100 μm). Percentage of SPRR2B$^+$ cells among total cells was estimated (n = 5 per group) (right). (D) Relative gene expression level of *Sprr2b* in mouse skin tissue (n = 5 per group). (E) Immunoblot analysis of extract of mouse skin tissue using IL36G and SPRR2B antibodies (left). Densitometric value of IL36G and SPRR2B bands normalized to HSP90 bands (right). All data are shown as mean ± SD. *P < 0.05, **P < 0.01 and ***P < 0.001 by one-way ANOVA with Tukey's test. NS, not significant.

DISCUSSION

AD contributes significantly to the global burden of skin disease due to its chronic and recurrent inflammatory nature (25). The pathophysiology of AD includes elevated IgE levels and proinflammatory cytokines, along with a compromised skin barrier that allows the penetration of allergens. These allergens activate Th2 cells, which release IL-4 and IL-13, leading to IgE production, mast cell activation, and the onset of AD symptoms (26). Keratinocytes play an important role by releasing cytokines and chemokines in response to external stimuli. Reducing pro-inflammatory mediators and hyperkeratosis is a promising therapeutic approach for AD (27). Non-ionizing electromagnetic fields (EMFs) are emerging as a promising alternative to conventional therapies (28). QMR is a novel approach that utilizes EMFs that produce distinct wave patterns at high frequencies in combination with low-intensity electric fields. Our study showed that QMR treatment significantly alleviated AD-like skin lesions in DNCB-induced mice, as evidenced by reduced mast cell and macrophage infiltration, decreased serum IgE levels, and restored epidermal integrity. RNA sequencing of mouse skin from each experimental group and RNA silencing experiments of TNF-α/IFN-γ-treated or DNCB-treated human keratinocytes identified IL36G, a gene associated with skin inflammation, and SPRR2B, a gene associated with keratinization, as key factors regulated by QMR. These genes were identified as novel and important contributors to inflammation and hyperkeratinization. A recent study showed that IL36G-deficient mice exhibit severe colonic inflammation compared to wild-type controls in a colitis model (29). In addition, emerging evidence shows a strong association between IL36G expression and psoriasis, a chronic inflammatory disease similar to AD that can activate various immune cells to trigger inflam-

matory cascades (30). IL36G also contributes to the recruitment of immune cells to the skin, leading to the characteristic inflammation observed in allergic contact dermatitis (31). Furthermore, recent studies have shown that variants associated with SPRR2B may have a significant impact on the development of atopic eczema and eczema-related asthma (24). In an allergen-induced asthma model, SPRR2B was reported to be upregulated in an IL-13-dependent manner, emphasizing its role in inflammation (32). Based on these findings, our study uncovered a novel therapeutic strategy using QMR, which we propose as a promising and effective alternative treatment for AD by suppressing IL36G and SPRR2B.

Although we proposed that QMR can alleviate AD by targeting IL36G and SPRR2B, the specific mechanisms were not fully elucidated in this study. We propose several hypotheses to support our positive experimental results. QMR may affect intracellular reactive oxygen species (ROS) pathways. It is well known that physical stimuli such as sonication or mechanical stress can alter intracellular ROS levels, which can affect various signaling pathways. QMR with high energy may also affect the state of molecules and associated enzymes, leading to changes in ROS-mediated intracellular pathways and decreased gene expression. QMR may also disrupt the binding of metals, such as zinc, within transcription factors or enzymes, causing their temporary dissociation from proteins (33). This disruption can alter transcriptional activity and signaling pathways, affecting protein-metal interactions and potentially reducing gene expression by inhibiting phosphatases. In addition, organic molecules within cells, with a variety of reversible bonds, can undergo changes in state or interactions due to QMR. Specifically, QMR may affect the redox state of organic molecules, leading to malfunction or loss of protein function. Our RNA sequencing data revealed changes in lipid metabolism, which can affect ROS signaling, in turn affecting the maintenance of organelles and the activation of immune cells. Consequently, these findings suggest that QMR has the potential to regulate the expression of IL36G and SPRR2B.

Recent research on IL36G therapeutic strategies has primarily involved antibody-based antagonists, highlighting the need for new inhibitors targeting IL36G. For example, the humanized monoclonal antibody spesolimab (BI 655130) blocks IL36 receptor (IL36R) signaling and is used to treat generalized pustular psoriasis (GPP) (34). Additionally, another high-affinity humanized monoclonal antibody, imsidolimab (ANB 019), targets IL-36R to inhibit cytokine signaling in GPP and other inflammatory skin diseases (35). However, the use of IL36R antagonists is associated with delayed wound healing due to excessive immune cell recruitment (36), and there are no commercially available small molecule-based IL36G therapies. On the other hand, no targeted therapies for SPRR2B currently exist, highlighting the need to develop novel inhibitors. Our study underscores the importance of advancing drug development efforts against these two targets to improve the treatment of AD.

We propose that the downregulation of IL36G and SPRR2B by QMR may function as a key factor in the regulation of signaling cascades that drive the expression of representative cytokines associated with type 2 inflammation, such as IL-4, IL-13, and IgE. Recent studies have shown that IL-36R- and IL-36γ-deficient mice fail to recover from acute intestinal injury due to significantly lower levels of IL-23, IL-22, and antimicrobial peptides. Additionally, IL-36R$^{-/-}$ and/or IL-36γ$^{-/-}$ mice exposed to an oxazolone-induced colitis model (a Th9 response associated with a predominantly Th2 response) showed improvement characterized by a decrease in mucosal IL-9-producing cells and an increase in T regulatory cells (37). Other studies have shown that QMR applied to the skin accelerates wound healing and has anti-inflammatory effects (38). QMR-based electrotherapy has also been utilized for many years to treat skin ulcers, with proposed mechanisms including reduced leukocyte infiltration (39). Furthermore, studies have shown that QMR application to the skin reduces the expression of matrix metalloproteinases, suggesting potential anti-inflammatory effects. Moreover, QMR application has been shown to significantly inhibit cancer cell cycle progression and reduce tumor volume, suggesting potential anticancer effects (40). Taken together, the QMR–IL36G/SPRR2B pathway plays an important role in anti-inflammation, suggesting important clinical and biological relevance in type 2 inflammation.

In summary, the application of QMR has been shown to significantly improve atopic markers, including atopic index scores, immune cell infiltration, cytokine expression, and skin structure damage, in a DNCB-induced AD animal model, with demonstrated efficacy comparable to or greater than DXM. Transcriptomic analysis showed that QMR potently suppressed immune responses and keratinization by inhibiting IL36G, a key initiator of inflammation, and SPRR2B, a novel regulator of keratinization. These findings provide strong evidence that QMR treatment alleviates atopic dermatitis by reducing IL36G and SPRR2B expression. This treatment results in improved skin symptoms, reduced inflammation, and modulation of key molecular pathways associated with AD. The use of QMR as a novel alternative therapy shows potential to manage AD, which is characterized by inflammation and hyperkeratosis of skin lesions, without the side effects associated with conventional therapies such as corticosteroids.

MATERIALS AND METHODS

Materials and methods are available in the supplemental material.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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