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Elucidating the potential pharmaceutical mechanism of *Gyejibokryeong-hwan* on rosacea using network analysis

Jundong Kim, MD, MSc[a], Kyuseok Kim, MD, PhD[b,*]

Abstract

Rosacea is a chronic erythematous disease with telangiectasia that affects the central area of the face. However, because of the ambiguity in the pathophysiology of rosacea, its treatment has not been clearly elucidated; therefore, new therapeutic options need to be developed. *Gyejibokryeong-hwan* (GBH) is widely used in clinical practice for various blood circulation disorders, including hot flushes. Therefore, we explored the potential pharmaceutical mechanism of GBH on rosacea and investigated the therapeutic points exclusive to GBH through comparative analysis with chemical drugs recommended in 4 guidelines for rosacea based on network analysis. The active compounds in GBH were identified, and the proteins targeted by these compounds and the genes related to rosacea were searched. Additionally, the proteins targeted by the guideline drugs were also searched to compare their effects. And the pathway/term analysis of common genes was conducted. Ten active compounds were obtained for rosacea. There were 14 rosacea-related genes targeted by GBH, with VEGFA, TNF, and IL-4, which were suggested as core genes. The pathway/term analysis of the 14 common genes revealed that GBH could potentially act on rosacea via 2 pathways: the "interleukin 17 signaling pathway" and the "neuroinflammatory response." Comparison and analysis of the protein targets between GBH and guideline drugs revealed that only GBH separately acts on the "vascular wound healing pathway." GBH has the potential to act on IL-17 signaling pathway, neuroinflammatory response and vascular wound healing pathway. Further studies are needed to determine the potential mechanism of GBH in rosacea.

Abbreviations: DL = drug-likeness, GBH = *Gyejibokryeong-hwan*, GO = gene ontology, IL = interleukin, KEGG = Kyoto encyclopedia of genes and genomes, MMP = matrix metalloproteinase, NF = nuclear factor, NK = natural killer, OB = oral bioavailability, PPI = protein-protein interaction, TCMSP = traditional Chinese medicine systems pharmacology database and analysis platform, TNF = tumor necrosis factor, TRP = transient receptor potential, UV = ultraviolet, VEGFA = vascular endothelial growth factor A.

Keywords: *Gyejibokryeong-hwan* (GBH), IL-17 pathway, network analysis, neuroinflammatory response, rosacea

1. Introduction

Rosacea is a chronic inflammatory vasodilatory skin disease that affects the central part of the face. It has a significant negative effect on the quality of life and is a risk factor for depression and anxiety.[1,2] The main symptoms of rosacea are erythema, telangiectasia, edema, papules, pustules, stinging, and hot flushes. Recently, the international prevalence of rosacea was reported to be approximately 5.5%.[3,4] To date, various hypotheses about the pathogenesis of rosacea have been proposed, such as an imbalance of the innate and adaptive immune systems, neurogenic inflammation, abnormal vascular-neurologic signaling, an imbalance between the skin/intestinal microbiota, and neuropsychiatric pathology.[4–8] Due to its complex pathophysiology, rosacea tends to be a deep-seated chronic disease, but the conventional therapeutic options mainly focus on temporary symptom-relieving effects.[9] Therefore, herbal medicines, which can act via multiple targets and pathways and exert overall regulatory and synergistic effects, could have advantages over conventional treatments in treating rosacea.[10]

Gyejibokryeong-hwan (GBH) (known as *Guizhi-fuling-wan* in China and *Keishibukuryo-gan* in Japan) is a prescription

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All data generated or analyzed during this study are included in this published article [and its supplementary information files]

Ethical statement: No ethical approval was required as this study did not involve human participants or laboratory animals.

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derived from the *Jinguiyaolue* (Synopsis of Prescriptions of the Golden Chamber) used to resolve blood stasis in the past and is consist of 5 medicinal herbs: *Cinnamomum cassia* J. Presl (Lauraceae), *Poria cocos* Wolf (Polyporaceae), *Paeonia suffruticosa* Andrews (Paeoniaceae), *Paeonia lactiflora* Pallas (Paeoniaceae), and *Prunus persica* Batsch (Rosaceae)[11] In the modern clinical practice of traditional Chinese medicine (TCM), blood stasis encompasses not only local static blood but also a wide range of blood circulation disorders. GBH is currently used to treat various vascular diseases, especially in relieving hot flushes.[12–15] As GBH has been reported to inhibit vascular permeability, prevent connective tissue hypertrophy, improve microcirculation, and help tissue regeneration, studies on cardiovascular, gynecological, and oncological diseases are being actively conducted.[16] For instance, studies on surface skin temperature have been actively conducted in Japan.[17–19] Considering these related studies and the clinical applications of GBH, it may have a potential therapeutic effect on rosacea.

The methodology of network analysis, which is based on systems biology, bioinformatics, and high-throughput histology, includes processes that investigate information regarding disease–gene–target protein-drug interactions and consequently discover potential underlying pathways.[20–22] Therefore, network analysis can properly reflect the distinct organic-integral perspective of TCM and is also suitable for studying diseases with complex pathophysiologies such as rosacea.[10] Therefore, this study utilized network analysis to decipher the novel insights into the underlying mechanism between GBH and rosacea.[23]

The primary purpose of this study was to identify the potential mechanisms involved in the therapeutic effects of GBH on rosacea through a network analysis. Secondly, through comparative analysis with chemical drugs recommended in the existing guidelines, we investigated the therapeutic points that GBH has exclusively.

2. Methods

We performed network analysis to explore the potential pharmaceutical mechanism of GBH on rosacea according to the workflow shown in Figure 1. The ethical approval or patient informed consent were not necessary because this study is a network analysis to explore the potential pharmaceutical mechanism of GBH on rosacea.

2.1. Determining the active compounds in GBH

To determine the active compounds of GBH, the web database Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; https://old.tcmsp-e.com/tcmsp.php) was used. TCMSP is a Chinese medicine-based database that contains more than 499 herbs, 29,384 active compounds, 3311 protein targets, and 837 related diseases. At the same time, it provides 12 absorption, distribution, metabolism, and excretion properties for each active compound.[24] The names of the 5 herbs of GBH were entered as search terms in Chinese, and screening was performed with the threshold of human oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18, as recommended by the TCMSP.[25] In particular, a DL value of 0.18 corresponds to the average DL index of the DrugBank database[26] and is also a universal and general cutoff criterion for OB and DL screening for pharmacological analysis.[27,28]

2.2. Target proteins of GBH compounds

The STITCH database ver. 5.0. (search tool for interactions of chemicals database, http://stitch.embl.de/) was utilized to confirm the proteins targeted by the active compounds of GBH. This database provides information on the interactions between specific compounds and proteins. Searches were performed using the names of the active compounds (e.g., kaempferol and stigmasterol) under the restriction conditions of *Homo sapiens*. Proteins that exceeded the high confidence score (0.700) were filtered and listed as a label of the HGNC gene symbol. A confidence score was assigned according to the predicted strength of the interaction, based on 5 main sources: genomic context prediction, high-throughput lab experiments, conserved co-expression, automated text mining, and previous knowledge in databases.[29] In general network pharmacology studies, screening is performed with the threshold of a high confidence score > 0.700.[25,30] Subsequently, Cytoscape software ver. 3.8.2. (Department of Bioengineering, University of California, San Diego, CA) was used to visualize the network relationship between the 5 herbs of GBH, active compounds, and target proteins.

2.3. Rosacea-related genes

Genetic information related to rosacea was collected using the GeneCards database ver. 5.5 (https://www.genecards.org/). The GeneCards database provides comprehensive information on human genes, diseases, mutations, proteins, cells, and biological pathways.[31] The database has been recently used to search for disease-related genes in various network pharmacology studies.[32,33]

The Medical Subject Headings database (MeSH Terms Database, https://www.ncbi.nlm.nih.gov/mesh/) was used for a more extensive search. The entry terms were checked by searching "Rosacea" in the MeSH database, and the results were as follows: Rosacea, rhinophyma, erythematotelangiectatic, papulopustular, "phymatous rosacea," "ocular rosacea," "granulomatous rosacea," and "acne rosacea." Accordingly, rosacea-related genes were identified in the Genecards database

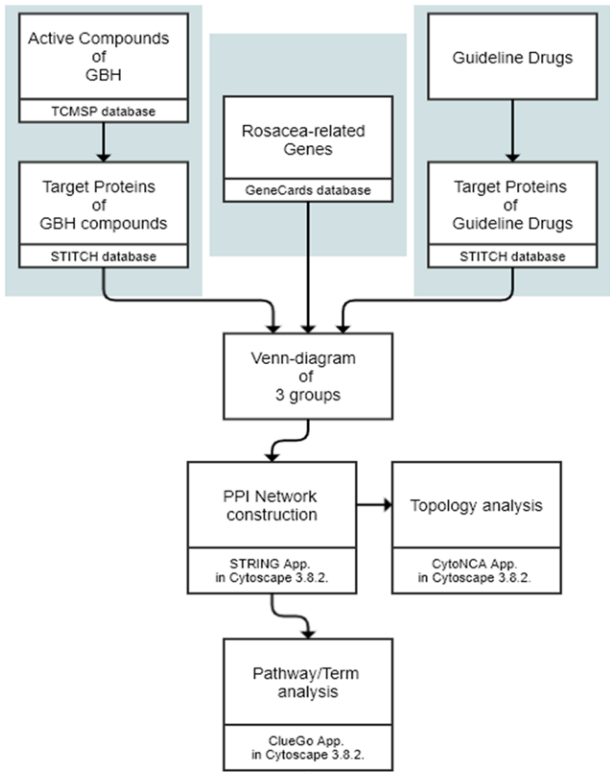

Figure 1. Flow chart of the study. GBH = Gyejibokryeong-hwan decoction, PPI = protein-protein interaction, TCMSP = Traditional Chinese Medicine Systems Pharmacology database and analysis platform, STITCH = search tool for interactions of chemicals database.

using each search term, and the extracted genes were listed in the form of a label of the HGNC gene symbol.

2.4. Target proteins of chemical ingredients mentioned in 4 guidelines

The proteins targeted by the currently recommended chemical drugs for rosacea were also investigated. Accordingly, the paper that compared and analyzed the 4 guidelines was referred to,[34] and all the recommended chemical ingredients were extracted. The 4 guidelines include the 2017 Global Rosacea Consensus (ROSCO), 2017 Swiss S1 guideline, 2016 Canada Clinical practice guideline, and the 2016 China Consensus.

The extracted chemical ingredients were divided into topical and systemic ingredients, and target proteins were searched using the STITCH database. All search conditions were the same as those for the GBH. The condition was limited to *Homo sapiens*, and the confidence score was set to >0.700. The search results were sorted in the form of a label for the HGNC gene symbol.

2.5. Establishment of a protein-protein interaction (PPI) network and topology analysis

A PPI network analysis was performed to confirm the interaction between the proteins common between GBH and rosacea. This process was performed using the StringApp ver. 1.7.0. (Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark) using Cytoscape software. This application is based on the STRING database, a representative database that integrates all direct (physical) and indirect (functional) data between proteins. This database collects and predicts PPI information through 4 main sources: co-expression analysis, signal sharing system analysis between genes, text mining, and computer prediction based on gene ontology (GO). A confidence score was assigned based on the 4 resources above, where a high score was >0.700, a medium score was between 0.400 and 0.700, and a low score was <0.400.[35]

In addition, CytoNCA application ver. 2.1.6. (School of Information Science and Engineering, Central South University, Changsha, China) was used to confirm the core genes in the PPI network. Screening was conducted using the following indicators that reflect the topological characteristics of the network: betweenness centrality, closeness centrality, degree centrality, eigenvector centrality, local average connectivity-based method, and network centrality. Nodes with all 6 indicators exceeding the average value were filtered and set as core genes.[36]

2.6. Gene ontology (GO) annotation and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

For the target proteins extracted above, ClueGo application ver. 2.5.8. (Laboratory of Integrative Cancer Immunology, Paris, France) was used to conduct GO functional annotation and KEGG signaling pathway enrichment analysis. First, an analysis of the overlapping proteins was performed to understand the therapeutic mechanism of GBH in rosacea. Second, each target gene list that overlapped with rosacea was analyzed and compared to confirm the differences between GBH and the drugs recommended by the guidelines.

The ClueGo application provides an analysis of the pathways/terms based on these 2 databases. In addition, it can provide a grouping function for similar pathways/terms.[37] The strength of this similarity was measured using chance-corrected kappa statistics. The terms/pathways with the highest significance within the functional group are representatively expressed.[38]

3. Results

3.1. Active compounds and target proteins of GBH

A total of 220 compounds were identified by searching guizhi (桂枝, Cinnamomi Ramulus, CR), 34 for Fuling (茯苓, Poria Cocos, PC), 55 for Mudanpi (牡丹皮, Moutan Cortex, MC), 119 for Chishao (赤芍, Paeoniae Rubra Radix, PRR), and 66 for Taoren (桃核, Persicae Semen, PS). After ADME screening with OB ≥ 30% and DL ≥ 0.18, active compounds were filtered into 7 for CR, 15 for PC, 11 for MC, 29 for PR, and 23 for PS.

Using the STITCH database, the target proteins of each compound were investigated. Seven compounds of CR targeted 19 proteins, 15 compounds of PC targeted 12 proteins, 11 compounds of MC had 29 target proteins, 29 compounds of PRR had 45 target proteins, and 23 compounds of PS had 13 target proteins. A list of active compounds and target proteins of each herb is provided in Supplemental Digital Content Table 1, http://links.lww.com/MD/I516 and Figure 2 shows the relationship between herbs, active compounds, and target proteins. Active compounds without significant targets were deleted from the network graph. Figure 2 shows a total of 104 nodes and 139 edges. Furthermore, the active compounds were sorted by degree index of network topology, and the results were as follows: β-sitosterol, hederagenin, baicalin, baicalein, ellagic acid, kaempferol, mairin, quercetin, taxifolin, and stigmasterol.

3.2. Rosacea-related genes

Rosacea-related genes were searched in the GeneCards database using MeSH terms, and a total of 599 genes were searched, among which 585 genes were searched with the term "rosacea," 26 genes with "rhinophyma," and 23 genes with "erythematotelagiectatic." All the above search results were combined, and 599 proteins were identified as rosacea-related genes (see Supplemental Digital Content Table 2, http://links.lww.com/MD/I517 which provides list of genes related to rosacea).

3.3. Chemical components of guideline drugs for rosacea

The ingredients of the chemical drugs used to treat rosacea mentioned in the 4 guidelines from 2016 to 2017 were integrated. The results are shown in Table 1, and the topical and systemic ingredients are sorted separately. Subsequently, the proteins targeted by the ingredients were searched in the STITCH database. Ninety target proteins for topical drugs and 76 target proteins for systemic drugs were identified. After integrating all the proteins and deleting the duplicates, 138 proteins were identified (see Supplemental Content Table 2, http://links.lww.com/MD/I517 which provides list of the target genes of GBH and the guideline drugs).

3.4. Analysis of the 14 common proteins between rosacea and GBH

3.4.1. PPI network construction and topology analysis. A Venn diagram of the 14 common proteins above was visualized (Fig. 3A). A PPI network was constructed based on a list of 14 common proteins of rosacea and GBH (Fig. 3B). The PPI network consisted of 14 nodes and 25 edges. Nodes represent target proteins and are connected with edges with an interaction intensity with a confidence score >0.700. The

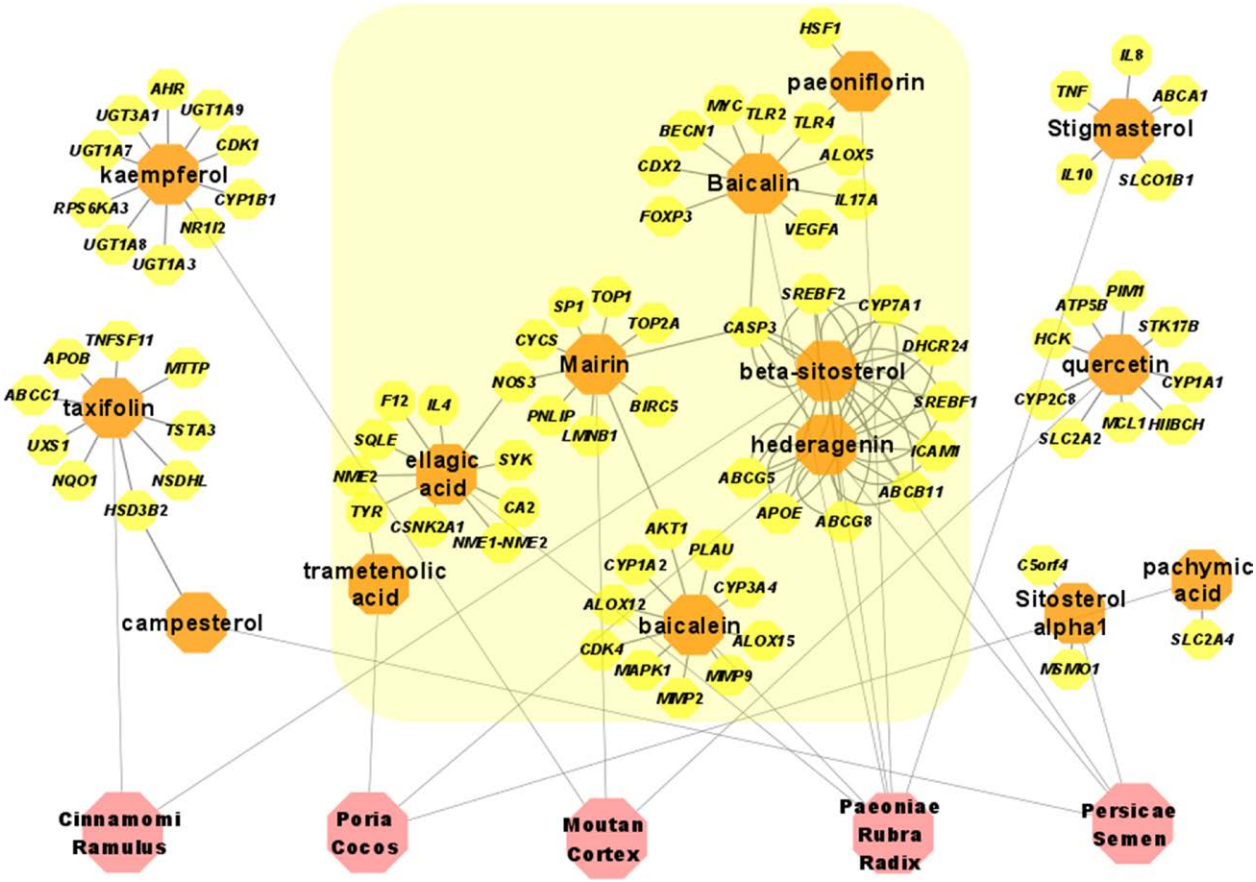

Figure 2. Herb-compound-target network of GBH as visualized using Cytoscape Ver. 3.8.2. Pink nodes represent herbs, orange nodes represent compounds, and yellow nodes represent target proteins. GBH = Gyejibokryeong-hwan decoction.

thicker the edge, the higher the confidence score. For the PPI network, additional analysis was performed according to the topological characteristics of the network to confirm the core genes (Table 2). The 3 genes that exceeded the average value in all 6 indicators were tumor necrosis factor (TNF), vascular endothelial growth factor A (VEGFA), and interleukin 4 (IL4).

3.4.2. GO annotation and KEGG pathway enrichment analysis using ClueGo Using the ClueGO app in Cytoscape, GO annotation and KEGG pathway analyses were performed on the 14 gene lists. A total of 24 BP terms were derived from the GO database, and 7 pathways were derived from the KEGG database. Accordingly, functional grouping was performed on total 31 pathways/terms, and 4 groups were identified: "IL-17 signaling pathway" (67.74%), "Regulation of neuroinflammatory response" (25.81%), "Negative regulation of cysteine type endopeptidase activity involved in apoptotic process" (3.23%), and "AGE-RAGE signaling pathway in diabetic complications" (3.23%) (Fig. 3C–E).

3.5. Analysis of rosacea and GBH (cluster #1) versus rosacea and guideline drugs (cluster #2)

3.5.1. GO annotation and KEGG pathway enrichment analysis using ClueGo. GO and KEGG analysis was performed using the list of proteins common between GBH and rosacea as cluster #1 (8 proteins) and the list of proteins common between guideline drugs and rosacea as cluster #2 (17 proteins). The overlapping proteins in both clusters were removed to confirm the differences between the 2 groups. A Venn diagram of 2 clusters is shown in Figure 4A.

One functional group, "vascular wound healing," appeared in GBH, and 4 subpathways/terms were suggested: "FceRI signaling pathway," "angiogenesis involved in wound healing," "vascular wound healing," and "positive regulation of tyrosine phosphorylation of STAT protein." For the guideline drugs, "regulation of neuroinflammatory response" (53.85%), "lipid and atherosclerosis" (35.9%), "regulation of oxidative stress-induced cell death" (5.13%), "response to vitamin A" (2.56%), and "regulation of myelination" (2.56%) were presented (Fig. 4B–D).

Table 1

Ingredients of the drugs recommended for rosacea in 4 guidelines.

Topical ingredients	Systemic ingredients
Brimonidine	Hydroxychloroquine
Azelaic acid	Doxycycline
Metronidazole	Macrolides
Tacrolimus, pimecrolimus	Carvedilol
Ivermectin	Isotretinoin
Benzoyl peroxide	Tetracycline
Erythromycin	Ampicillin
Clindamycin	Azithromycin
Permethrin	Tinidazole
Sulfur lotion	Ivermectin
Cyclosporine	Zinc sulfate
Retinoids (tretinoin, isotretinoin, acitretin, etretinate, adapalene, tazatotene)	Metronidazole

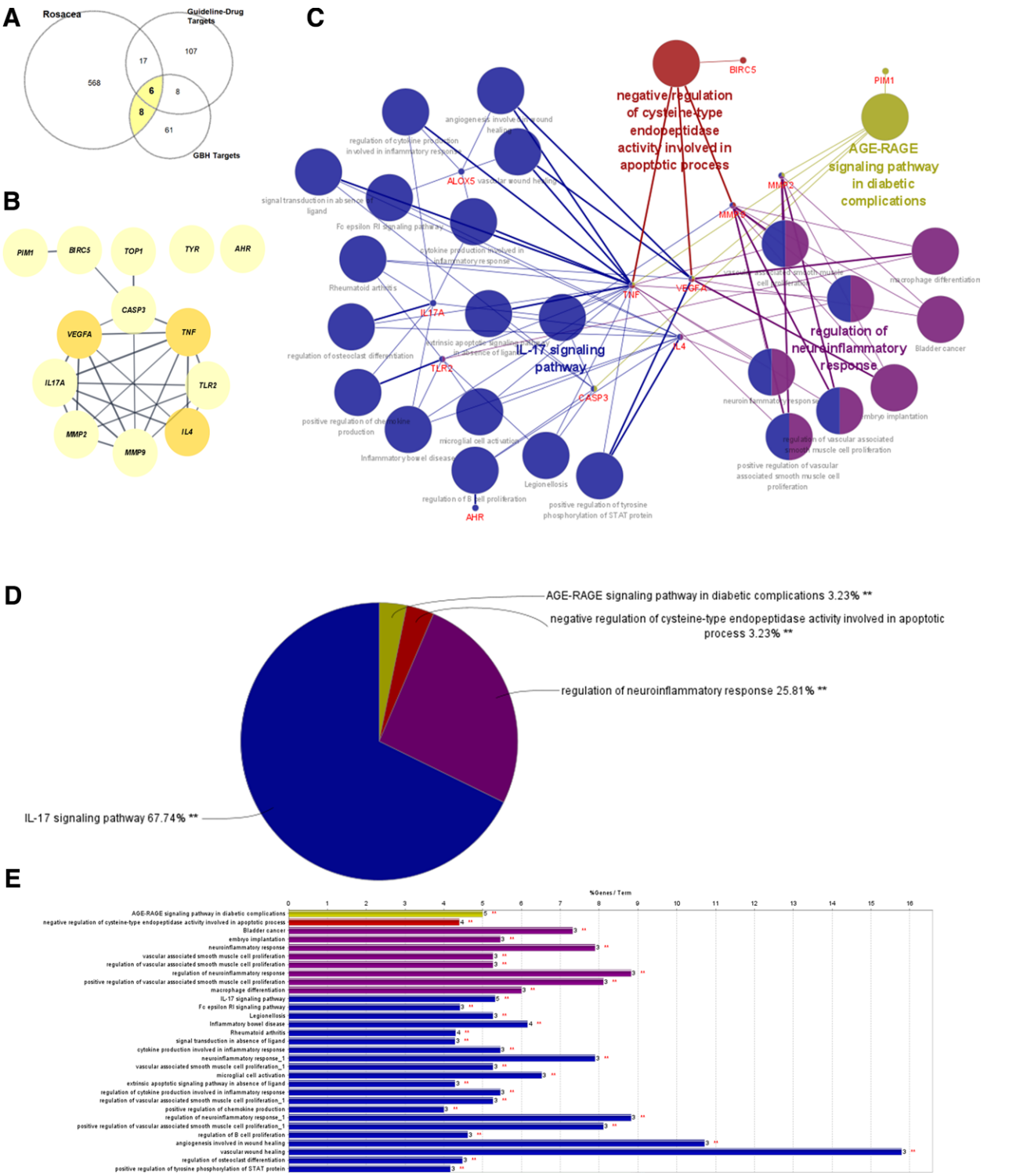

Figure 3. PPI network construction and GO annotation and KEGG pathway enrichment analysis of the 14 common proteins between rosacea and GBH. Venn diagram of target proteins and rosacea-related genes (A). The yellow-orange area represents the overlap between rosacea and GBH. The 14 proteins in the yellow-orange area were further analyzed. Protein-protein interaction network graph of the 14 proteins in the yellow-orange area of the Venn diagram (B). Orange nodes are the core genes. Network of 14 targets proteins and related pathways by ClueGo ver 2.5.8 in Cytoscape (C), percentage of terms per group (D), and percentage of genes per term (E). GBH = gyejibokryeong-hwan decoction, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, PPI = protein-protein interaction.

4. Discussion and conclusion

The potential pharmaceutical mechanism of GBH on rosacea, which is clinically used and studied for various vascular diseases and skin diseases, was explored in silico. First, the herb–compound–target protein network of GBH was identified and visualized. Active compounds with 2 or more protein targets in the network were summarized in order of highest degree as follows: β-sitosterol, hederagenin, baicalin, baicalein, ellagic acid, kaempferol, mairin, quercetin, taxifolin, stigmasterol. In particular, β-sitosterol, kaempferol, and stigmasterol are

Table 2

Identification of core genes using CytoNCA ver. 2.1.6.

	Degree	Eigenvector	LAC	Betweenness	Closeness	Network
TNF*	7^	0.427533^	4^	19.73333^	0.295455^	6.25^
IL17A	6^	0.391941^	4^	2.4	0.270833^	5.35^
VEGFA*	6^	0.376207^	3.333333^	15.33333^	0.288889^	4.35^
MMP9	6^	0.383275^	3.666667^	9.733334	0.282609^	4.85^
CASP3	5^	0.231448^	1.2	52^	0.282609^	1.5^
MMP2	5^	0.347684^	3.6^	0.4	0.26^	4.5^
TLR2	5^	0.33411^	3.2^	2	0.265306^	4^
IL4*	5^	0.287437^	2^	20.4^	0.265306^	2.5^
BIRC5	2	0.043557	0	20^	0.240741^	0
TOP1	1	0.042097	0	0	0.232143	0
ALOX1	1	0.052262	0	0	0.220339	0
PIM1	1	0.007965	0	0	0.203125	0
AHR	0	0	0	0	0.071429	0
TYR	0	3.45^	0	0	0.071429	0
Average	3.571429	0.208965	1.785714	10.14286	0.232158	2.378571

*means the core genes. Values with ^ are over the average value.

LAC = local average connectivity-based method.

representative plant sterols (phytosterols) that have been reported to have anti-inflammatory and antipruritic effects. In addition to its anticancer, antioxidant, antiviral, and anti-inflammatory effects,[39] β-sitosterol upregulates the synthesis of total ceramide and hyaluronic acid to prevent skin aging. It also reduces the serum levels of histamine, IgE, and IL-4 and downregulates the production of thymic stromal lymphopoietin, thereby exerting an antipruritic effect.[39–41] Kaempferol has been studied in various skin diseases, including atopic dermatitis, psoriasis, scleroderma, skin cancer, and burn-induced skin injury. It has been shown to reduce the release of pro-inflammatory cytokines, such as IL-6, IL-17A, TNF-α, and nuclear factor (NF)-κB.[42–46] Stigmasterol has also been reported to have anticancer and antiallergic effects.[47,48] However, upon interaction with VEGF, kaempferol has been reported to have an angiogenesis-enhancing effect; conversely, stigmasterol has an inhibitory effect on angiogenesis. Therefore, it is expected that GBH will have a regulatory action on angiogenesis; however, further research is needed to elucidate the precise effects.[49,50]

Hederagenin has a strong anti-inflammatory effect. It inhibits the expression of various pro-inflammatory substances, such as NO, PGE2, TNF-α, IL-1β, and IL-6, and inhibits the NF-κB pathway.[51] Baicalin has also been reported to inhibit ultraviolet (UV) radiation-induced and *Propionibacterium acnes*-induced skin inflammation, along with potent anti-inflammatory action through the downregulation of the NF-κB/mitogen-activated protein kinase signaling pathway.[51,52] Baicalin also has an anti-inflammatory action against UV radiation and oxidative stress, which are the main aggravating factors of rosacea. Moreover, it inhibits the expression of matrix metalloproteinase (MMP)-1 in fibroblasts in the dermis via the transient receptor potential (TRP) vanilloid 1-Ca-extracellular signal-regulated kinase pathway, which affects the granulomatous change of rosacea.[53] Ellagic acid also has anti-inflammatory and IL-6 inhibitory effects.[11] In rosacea, IL-6 acts on Th17 cells to stimulate the secretion of IL-17, which can induce chronic inflammation.[54] In addition, quercetin prevents the overproduction of reactive oxygen species.[11] It has also been reported to have anti-inflammatory effects on various skin inflammatory diseases, such as psoriasis and atopic dermatitis.[55,56]

Next, a Venn diagram analysis was performed. Fourteen proteins were the common denominators of GBH and rosacea. A network graph was created based on these 14 proteins, and a topology analysis was performed to identify the core genes. The 3 core genes were VEGFA, TNF, and IL4.

In the development of blood vessels and vascular network patterning, VEGF, fibroblast growth factor, and angiopoietin-1 and -2 are known to play an important role as signaling molecules.[57] In particular, VEGFA has been reported to regulate most vascular endothelial cell responses, including proliferation, migration, and permeability control. Physiologically, VEGFA maintains homeostasis and accelerates wound repair. However, pathologically it induces the progression of angiogenesis-related diseases.[58] In patients with a reddened face, one of the clinical symptoms of rosacea, it was reported that VEGFA was overexpressed in the granular layer and the stratum corneum of keratinocytes.[59] Elevated levels of tissue VEGFA may contribute to an increased vascularity.[60] In addition, VEGFA-induced angiogenesis responds more strongly to UVB exposure, which means that VEGFA can be a mediating factor when UV radiation aggravates the symptoms of rosacea.[61]

TNF is a key mediator that regulates the inflammatory response under physiological and pathological conditions.[62] In rosacea, TNF is involved in amplifying inflammation, particularly in papule and pustule formation. TNF is also involved in localized fever in the lesion, the sensitization of LRR and PYD domain-containing protein 3 (NALP3), activation of IL-1β and NF-κB-related proteins, and angiogenesis, leading to chronic inflammation.[63]

IL-4 is a growth factor derived from T cells, innate lymphocytes (natural killer [NK] cells), and myeloid cells (basophils and mast cells). It acts on B cells and is a key cytokine for the type 2 immune response.[64] It is mainly studied in allergic diseases, including atopic dermatitis and asthma, and is also involved in chronic inflammatory diseases, such as psoriasis. However, the direct association between IL-4 and rosacea has yet to be precisely defined. However, in a histological analysis of hypertrophic scars of atopic dermatitis, it was confirmed that the expression of IL-4, transforming growth factor-β, and insulin-like growth factor-1R increased. Therefore, there is a need to study the expression level of IL-4 in hypertrophic lesions of patients with rosacea, as well as the preventive effect of hypertrophic changes in GBH treatment.

A previous study found that the blockade of IL-4 causes rosacea. A 67-year-old woman with atopic dermatitis was treated with dupilumab, a biologic agent that blocks the IL-4/IL-13 signaling pathway, and transient symptoms of rosacea occurred.[65] The authors hypothesized that the blockade of the Th2 pathway by dupilumab induced the proliferation of *Demodex*, thereby increasing IL-17-mediated inflammation. Hence, there is a need to clarify the effects of GBH on IL-4.

In summary, VEGFA is involved in angiogenesis and the vasculature in the pathology of rosacea, as it can induce vascular

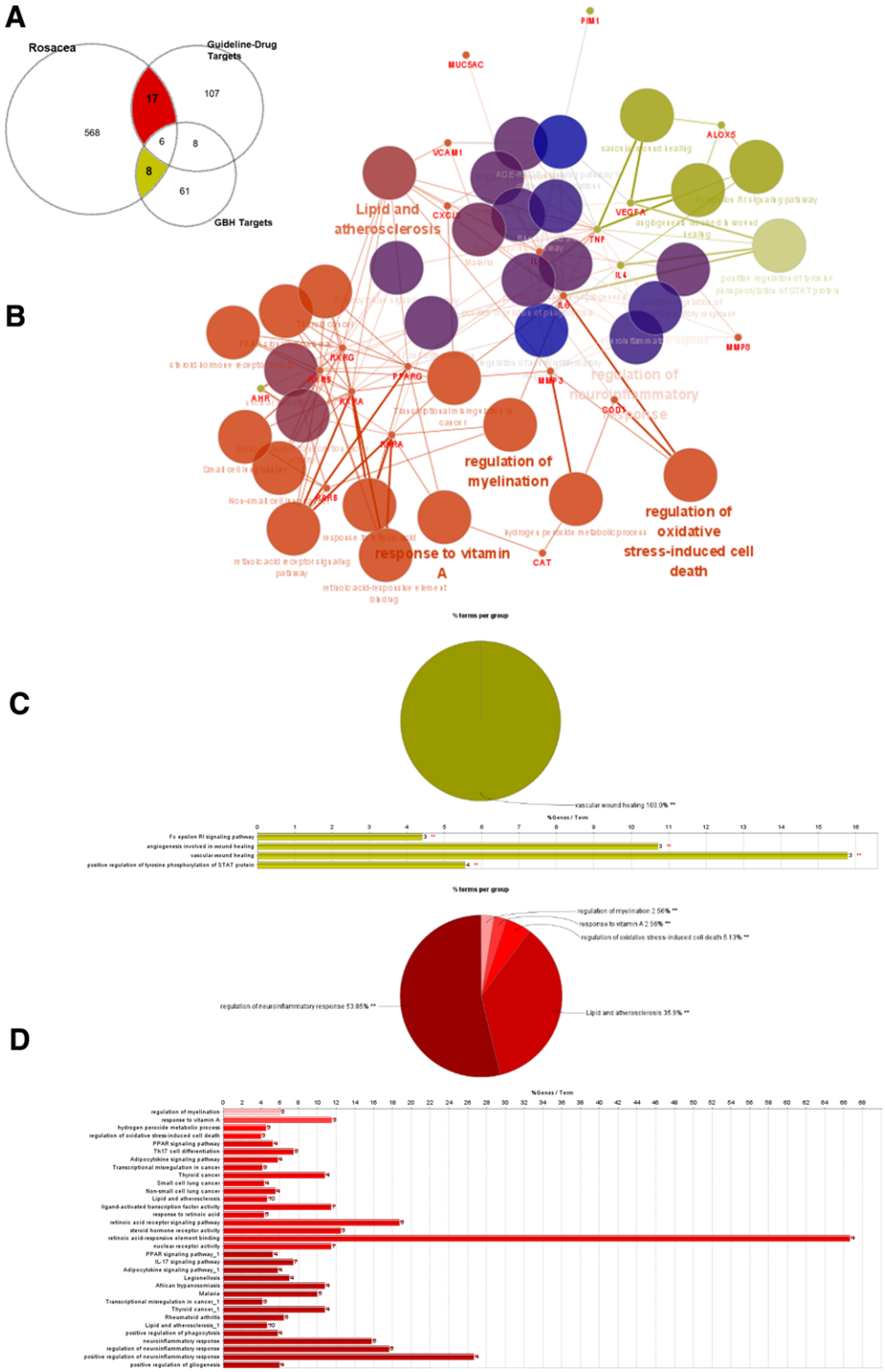

Figure 4. Analysis of rosacea and GBH versus rosacea and guideline drugs. Venn diagram of rosacea-related genes, guideline drug targets, and Gyejibokryeong-hwan (GBH) targets (A). The red area represents the overlap between rosacea and the targets of the guideline drugs. The green area represents the overlap between rosacea and the targets of GBH. Gene-pathway network of 8 target proteins from GBH and rosacea and 17 proteins from the guideline drugs and rosacea (B), functionally grouped pathways/terms of GBH target proteins only (C), and functionally grouped pathways/terms of guideline drug target proteins only (D).

symptoms. TNF is involved in the overall inflammatory process, and inflammatory symptoms, such as the formation of papules and pustules, can be affected. IL-4 may be associated with hypertrophic changes in rosacea; however, further studies are needed to confirm this.

Furthermore, we investigated how the 14 proteins common between rosacea and GBH are related. The 31 pathways/terms uncovered were functionally grouped using ClueGo. Four groups were prepared: IL-17 signaling pathway, regulation of neuroinflammatory response, negative regulation of cysteine-type endopeptidase activity involved in the apoptotic process, and AGE-RAGE signaling pathway in diabetic complications.

Various studies have been conducted on the pathological mechanisms of rosacea. In particular, abnormalities of innate

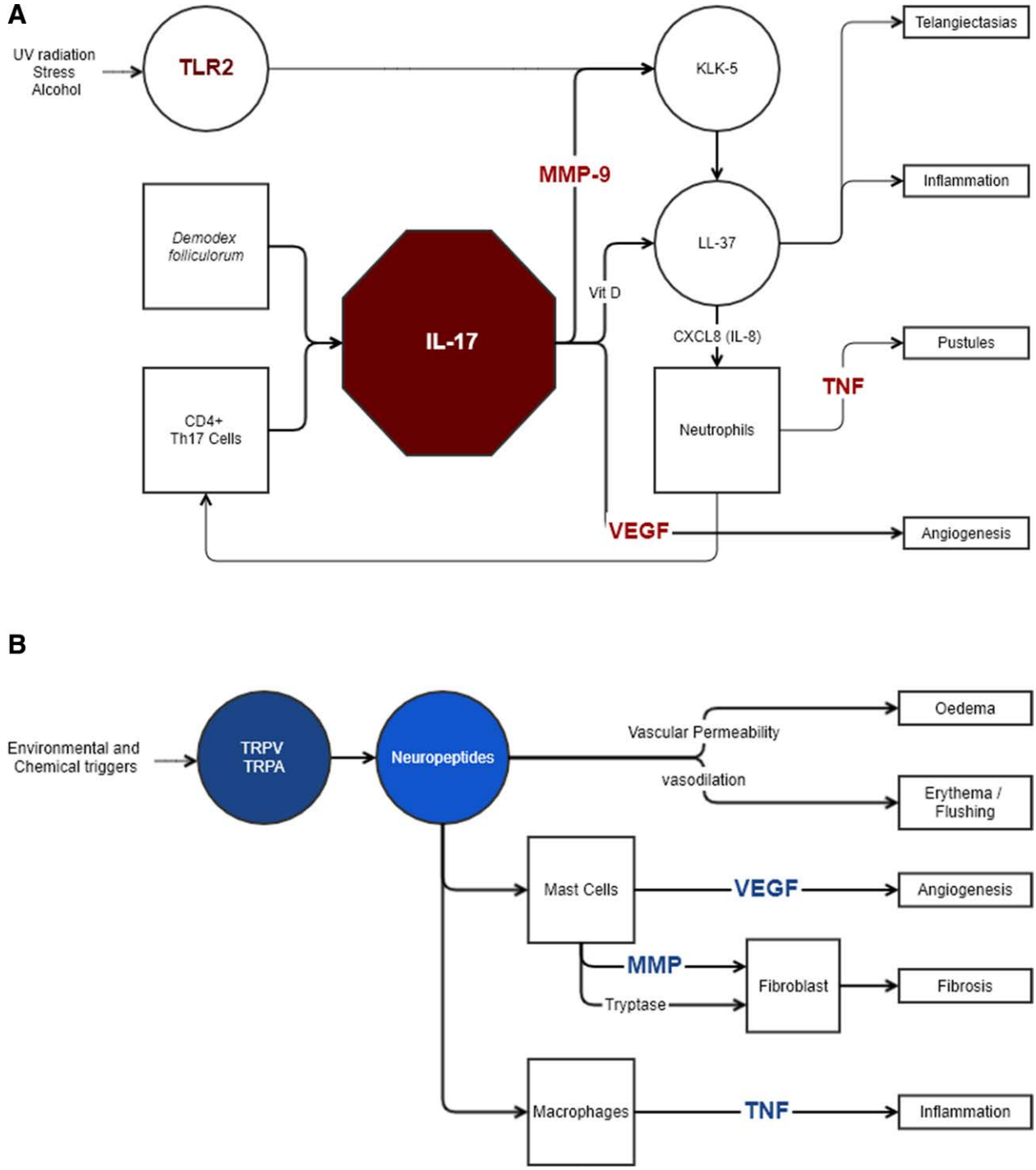

Figure 5. The role of the IL-17 pathway in rosacea (A) and the neuroinflammatory response in rosacea (B). Dark red proteins are the potential targets of Gyejibokryeong-hwan (GBH). Blue proteins are the potential targets of GBH. IL = interleukin, KLK = kallikrein, MMP = matrix metalloproteinase, Th = helper T cells, TLR = toll-like receptor, TNF = tumor necrosis factor, VEGF = vascular endothelial growth factor, Vit = Vitamin.

and adaptive immune function, neurogenic inflammation, and vascular dysregulation are the most important pathologies discussed.[3] Interestingly, the potential mechanism of GBH reported in this study is also consistent with these 3 pathologies.

The IL-17 signaling pathway plays a key role in rosacea because it greatly contributes to the hyperactivity of the innate and adaptive immune functions.[66] It also plays a role in the development of angiogenesis.[66] IL-17 is a cytokine produced mainly by T helper (Th) 17 cells. However, it has also been found to be produced by various neutrophils, including macrophages, dendritic cells, and NK cells, which are noticeable in papulopustular rosacea. Its secretion can be stimulated by UV radiation, stress, and *Demodex*.[66] IL-17 produces MMP-9, which triggers the activation of Kallikrein (KLK)-5, which in turn activates the immune response by generating LL-37. Alternatively, LL-37 can be generated directly through vitamin D. LL-37 exacerbates the inflammatory response through cytokine production, chemotaxis, and telangiectasia. In addition, LL-37 stimulates the secretion of CXCL8 (IL-8), which facilitates the recruitment of neutrophils, thus inducing pustular symptoms. These recruited neutrophils secrete IL-17 to form a chronic inflammatory circle.[66,67] In addition, IL-17 stimulates

the activation of VEGF-induced angiogenesis. In summary, IL-17 activates the immune system in rosacea, playing a major role in the inflammatory response, vasodilation, and pustule formation, and is a cytokine partially involved in angiogenesis. The potential targets of GBH, IL17A, TLR2, MMP-9, TNF, and VEGFA are highlighted in red in Figure 5A. In addition, ALOX5, CASP3, IL4, and MMP2 are also involved in the IL-17 signaling pathway.

It appears that GBH may also be involved in the neurogenic inflammatory response of rosacea. The starting point of the neurogenic inflammatory response of rosacea is the increased expression of TRP ion channels in sensory neurons. An increased expression of TRP channels elevates the secretion of various neuropeptides, such as substance P (SP).[68] These neuropeptides directly dilate blood vessels and increase vascular permeability, causing edema, erythema, and a burning sensation.[69] Neuropeptides also stimulate mast cells and fibroblasts to secrete various cytokines, such as VEGF, MMP, and tryptase, leading to symptoms such as angiogenesis and skin fibrosis.[69] The proteins corresponding to the potential targets of GBH in this neuroinflammatory response are highlighted in blue in Figure 5B.

Taken together, the 2 main categories in which GBH acts in rosacea are the IL-17 signaling pathway and the neuroinflammatory response, the main target proteins of which were identified. Metronidazole is a representative drug currently used for the treatment of rosacea. It inhibits the action of IL-17, CXCL8, and IL-6, and has an antibiotic effect against *Demodex folliculorum*. Doxycycline is also frequently used to treat rosacea as it mainly acts by inhibiting MMP-9, KLK-5, and IL-8. Similarly, ivermectin inhibits IL-8, LL-37, and TNF.[66] In other words, the underlying mechanisms of GBH and chemical drugs partially overlap or act independently. In addition, GBH is thought to be involved in various pathways compared to chemical drugs. Therefore, it is expected that the therapeutic effect on rosacea will increase when a combination of GBH and chemical drugs is administered in patients who do not show sufficient effect when using chemical drugs or GBH alone. Thus, it is necessary to conduct further studies to verify the potential synergistic effects of the co-administration of GBH and chemical drugs.

Next, we attempted to identify the mechanisms exclusive to GBH. To this end, the protein targets of GBH and guideline drugs were compared and analyzed. The functional pathways/term in GBH revealed "vascular wound healing," with the major relevant proteins being VEGFA, TNF, IL4, and ALOX5. The subgroups of "vascular wound healing" were "angiogenesis in wound healing," "vascular wound healing," "positive regulation of tyrosine phosphorylation of STAT protein," and the "FcεRI signaling pathway." All of these subgroups appear to be consistently associated with the angiopathy of rosacea, a specific mechanism only related to GBH. Recently, Scharschmidt et al proposed a new subtype of rosacea, "neurogenic rosacea.[70]" Patients with this type have prominent vasomotor and neuropathic symptoms and mainly complain of hot flushes, burning sensations, vasodilation, and telangiectasia. However, patients with this disease do not respond well to existing drug treatments. This might be because, as described above, existing guidelines do not directly target the angio-pathology of rosacea. In clinical practice, neuroleptic agents (such as pregabalin and gabapentin), tricyclic antidepressants, and duloxetine have been tested in patients with neurogenic rosacea.[71] However, these drugs cannot be prescribed for long periods of time due to safety concerns, where potential that adverse effects, including psychiatric symptoms, might occur. However, as found in this study, GBH has the potential to act on the angiopathy of rosacea. In addition, recent experimental studies have reported the antidepressant effects of GBH.[72] Therefore, if the clinical effect of GBH on neurogenic rosacea has been sufficiently studied, it is expected to be actively utilized as an appropriate alternative drug for treating neurogenic rosacea.

This study was an in silico study that utilized various databases. Accordingly, in the search process, there may be missing or abbreviated content due to the heterogeneity of the databases, search terms, and languages used. In addition, only simple correlations were reflected without revealing specific details regarding up/downregulation. Therefore, a limitation of this study is that the results can only suggest a potential mechanism. Thus, further studies will be needed to prove the hypotheses proposed here.

This study elucidated the potential underlying mechanism of GBH on rosacea. GBH could potentially act on rosacea via the "IL-17 pathway" and the "neuroinflammatory response" in a comprehensive pharmacological manner. In addition, GBH may be separately involved in "vascular wound healing," which is not targeted by the existing chemical drugs for rosacea treatment. These therapeutic points of GBH could provide a potential treatment option for neurogenic rosacea, a newly proposed subtype of rosacea that do not respond well to existing drug treatments. However, to verify these results, more research will need to be carried out in the future.

Author contributions

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Project administration: Kyuseok Kim.
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