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Exploring Co-expression Modules-Traits Correlation through Weighted Gene Co-expression Network Analysis: A Promising Approach in Wound Healing Research

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Abstract

Background: The skin is the biggest organ in the body and has several important functions in protection and regulation. However, wound development can disrupt the natural healing process, leading to challenges such as chronic wounds, persistent infections, and impaired angiogenesis. These issues not only affect individuals' well-being but also pose significant economic burdens on healthcare systems. Despite advancements in wound care research, managing chronic wounds remains a pressing concern, with obstacles such as persistent infection and impaired angiogenesis hindering the healing process. Understanding the complex genetic pathways involved in wound healing is crucial for developing effective therapeutic strategies and reducing the socio-economic impact of chronic wounds. Weighted Gene Co-Expression Network Analysis (WGCNA) offers a promising approach to uncovering key genes and modules associated with different stages of wound healing, providing valuable insights for targeted interventions to enhance tissue repair and promote efficient wound healing.

Methods: Data collection involved retrieving microarray gene expression datasets from the Gene Expression Omnibus website, with 65 series selected according to inclusion and exclusion criteria. Preprocessing of raw data was performed using the Robust MultiArray Averaging approach for background correction, normalization, and gene expression calculation. Weighted Gene Co-Expression Network Analysis was employed to identify co-expression patterns among genes associated with wound healing processes. This involved steps such as network construction, topological analysis, module identification, and association with clinical traits. Functional analysis included enrichment analysis and identification of hub genes through gene-gene functional interaction network analysis using the GeneMANIA database.

Results: The analysis using WGCNA indicated significant correlations between wound healing and the black, brown, and light green modules. These modules were further examined for their relevance to wound healing traits and subjected to functional enrichment analysis. A total of 16 genes were singled out as potential hub genes critical for wound healing. These hub genes were then scrutinized, revealing a gene-gene functional interaction network within the module network based on the KEGG enrichment database. Noteworthy pathways such as MAPK, EGFR, and ErbB signaling pathways, as well as essential cellular processes including autophagy and mitophagy, emerged as the most notable significant pathways.

Conclusion: We identified consensus modules relating to wound healing across nine microarray datasets. Among these, 16 hub genes were uncovered within the brown and black modules. KEGG enrichment analysis identified co-expression genes within these modules and highlighted pathways most closely associated with the development of wound healing traits, including autophagy and mitophagy. The hub genes identified in this study represent potential candidates for future research endeavors. These findings serve as a stepping stone toward further exploration of the implications of these co-expressed modules on wound healing traits.

Keywords: Wound Healing, Gene Expression, Network Analysis, Autophagy, Mitophagy

Conflicts of Interest: *None declared*
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↑What is "already known" in this topic:
The current understanding of this field encompasses the recognition of several essential functional genes that contribute to skin wound healing. These genes have been divided into groups based on their distinct co-expression patterns and their strong association with specific traits that are critical for the successful progression of wound healing.

→What this article adds:
This study identifies the key genes and modules which are crucial for the wound healing process. These genes and the things they produce may be useful targets for intervention in people who present with difficulties. We report 16 hub genes within specific modules, shedding light on their importance in promoting efficient wound healing.

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Introduction

The skin is the largest organ in the human body and serves a variety of essential functions, including controlling body temperature, storing energy, and acting as a protective barrier against external threats such as bacteria, viruses and physical injury (1). However, the natural healing process can be disrupted by factors such as wound severity, leading to challenges like chronic wounds, persistent infection, and impaired angiogenesis. Persistent infection can lead to prolonged inflammation, which can impede the normal progression of the wound-healing process. Impaired angiogenesis, on the other hand, can result in inadequate blood supply to the wound site, leading to poor oxygen and nutrient delivery, which are essential for the healing process (2, 3). Skin wounds present a multitude of challenges for individuals and society, including the economic burden of treatment and the impact on individuals' ability to work and participate in daily activities. The cost of treating chronic wounds, in particular, is substantial and can have a significant impact on healthcare systems and society as a whole (4). In the United States alone, the annual cost of treating chronic wounds is estimated to exceed billions of dollars, encompassing direct medical expenses and indirect costs like loss of productivity and decreased quality of life for affected individuals (5). Approximately 6.5 million Americans experience severe wounds, which equates to more than $25 billion in yearly medical expenses (6). It is anticipated that these rates will climb significantly in the future due to the dramatic growth in the prevalence of diabetes, obesity, and chronic infections, among other conditions (7). Despite significant efforts and breakthroughs in wound healing research and product development, effectively managing chronic and full-thickness wounds remains a significant challenge with obstacles like persistent infection, ongoing inflammation, and impaired angiogenesis hindering the healing process. In addition, damaged skin offers a fertile habitat for the development and multiplication of germs in deep wounds, and the loss of the vascular bed may result in chronic damage or even the loss of the skin and its underlying structures (8-10).

Research efforts worldwide are focused on understanding the healing processes of skin lesions and wounds, leading to significant breakthroughs in treating skin issues. Studies have revealed changes in gene expression throughout the wound-healing process, with researchers using diverse data sources and advanced computational methods to gain insights into the role of genes in cancer development (11-14). Certain drugs have been found to promote early wound healing and exert a positive influence on the overall wound-healing process (15). Enhancing our understanding of the wound-healing process is essential for healthcare professionals to provide effective treatment with ongoing research into the mechanisms holding the potential to advance wound care therapies and enhance outcomes for individuals with chronic wounds (16, 17). By persistently deepening our comprehension of the wound healing process, we can work towards developing more effective strategies for treating skin wounds and reducing the socio-economic impact of chronic wounds on individuals and society as a whole.

However, gaps persist in our understanding of the genetic pathways involved in wound healing, particularly in the intricate network of genes and their interactions across different healing stages (18). Weighted gene co-expression network analysis (WGCNA) emerges as a promising approach to address these knowledge gaps by identifying co-expressed gene modules crucial for various phases of wound healing, from inflammation to tissue repair and remodeling (18, 19). WGCNA offers a systematic approach to bridge the knowledge gaps by identifying co-expressed gene modules that may play pivotal roles in different stages of wound healing, from inflammation and tissue repair to remodeling (19).

In line with the highlighted challenges in wound healing and the potential of WGCNA, this study aims to bridge the existing knowledge gaps in gene expression during wound healing stages. By employing WGCNA techniques, the goal is to identify and characterize specific gene modules crucial in various phases of wound healing. This research seeks to unravel the intricate genetic mechanisms underlying efficient tissue repair and ultimately contribute to the development of targeted therapeutic interventions that promote accelerated and effective wound healing.

Methods

Data collection and quality control

This research collected microarray gene expression datasets from the Gene Expression Omnibus (GEO), NCBI website (https://www.ncbi.nlm.nih.gov) (20). The datasets underwent manual screening to identify those containing data on skin and oral wound healing in human subjects. Through manual screening, 65 series (out of the initial 7500 series) were short-listed. Subsequently, these series were subjected to quality control and assessed against a predefined set of inclusion and exclusion criteria. Ultimately, only 9 series (from 73 patients and 42 healthy controls) assembly of the criteria were selected and included in the study. Our inclusion criteria comprised human wound healing participants, and a high-throughput microarray was eligible for inclusion. Exclusion criteria included research on non-human samples, samples with therapy or another concomitant disease, cell lines, and non-blood samples. Selected series were deposited into the database under accession numbers GSE21648, GSE30355, GSE7890, GSE28914, GSE63107, GSE11919, GSE440, GSE26487,

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Table 1. Characteristics of datasets

No.	Tissue type	GEO ID	Platform	Participant group	Sample Size
1	Skin and oral	GSE21648	Affymetrix, GPL96	Patients	15
2	Skin	GSE30355	Affymetrix, GPL570	Patients	10
3	Skin	GSE7890	Affymetrix, GPL570	Patients	10
4	Oral	GSE28914	Affymetrix, GPL570	Patients	8
5	Skin	GSE63107	Affymetrix, GPL570	Patients	30
6	Skin	GSE11919	Affymetrix, GPL570	Healthy Controls	9
7	Skin	GSE440	Affymetrix, GPL8300	Healthy Controls	5
8	Skin	GSE26487	Affymetrix, GPL8300	Healthy Controls	10
9	Skin	GSE427	Affymetrix, GPL8300	Healthy Controls	18

and GSE427 (12, 21-28) which are shown in details in Table 1 (29). It's essential to acknowledge that while this series provided valuable insights, the sample size was inherently limited by the available data within the specified criteria. The constraints imposed by the inclusion/exclusion criteria and the limited number of studies meeting these stringent conditions significantly influenced the final sample size utilized for our analysis.

The raw data underwent preprocessing in R using the Robust MultiArray Averaging (RMA) approach (30), which comprised background correction, normalization, and expression calculation (version 1.38.0). RMA is known for its effectiveness in correcting background noise, normalizing data, and accurately calculating gene expression levels. Its robust nature makes it a preferred method for preprocessing microarray data, as it reduces technical artifacts and improves the reliability and comparability of gene expression measurements across samples (31).

WGCNA network construction

Weighted Gene Co-Expression Network Analysis (WGCNA) was utilized to identify genes relevant to wound healing processes. The process involved key steps:

Data Collection and Preprocessing: The data from 'Group' (wounded and healthy controls) without outliers was gathered. Probes lacking consistent gene symbols were removed, and gene expression values were calculated by averaging probes mapped to the same gene symbol.

Network Construction and Topological Analysis: Soft-thresholding power analysis determined the appropriate power value for network construction. Adjacencies were calculated, and a consensus Topological Overlap Matrix (TOM) was created to represent gene interconnectedness. TOM helps identify groups of genes' interconnectedness (18).

Module Identification and Analysis: Modules of co-expressed genes were identified using the 'cutreeDynamic' function, grouping genes based on their expression patterns. Eigengenes (MEs) representing module expression profiles were computed and correlated with consensus modules (18). Modules with high correlation (0.75) were merged to create larger modules.

Association with Clinical Traits: The relationship between clinical traits and gene significance (GS), as well as module membership (MM) was explored using correlation analysis and P-value computation. GS measures the correlation between gene expression and a specific (18). While MM indicates how well a gene fits into a module. Genes with high MM values are tightly interconnected within their module, indicating their strong association with the module's expression pattern (18).

Focus on Wound Healing-Related Modules: A specific investigation into modules closely linked to wound healing was conducted by identifying modules significantly associated with this process. These modules were then connected back to the broader consensus modules, providing insights into their interrelationships within the gene network.

Functional analysis

We performed functional enrichment analysis using the clusterProfiler R package (32), which is specifically designed for uncovering the biological significance of gene clusters or sets. The aim of this analysis was to identify and highlight enriched biological pathways, functions, and ontologies associated with the candidate genes obtained from WGCNA (32).

To visualize the significantly enriched KEGG keywords, dot plots were used, and only those with an adjusted *P*-value of < 0.05 (based on the Benjamin and Hochberg ('BH') method) were considered significant.

Identification of Hub Gene and the gene-gene functional interaction network

Hub genes are characterized by a high degree of correlation within specific modules. Typically, these genes exhibit a geneModuleMembership >0.80 and a geneTraitSignificance >0.20 in absolute value. Using these values, hub genes related to wound healing were detected. The GeneMANIA database was also used to create a gene-gene functional interaction network for the hub genes. GeneMANIA is a database that is used to create hypotheses about gene function, assess gene lists, and select gene priorities for functional research (33). It may be used to uncover protein-protein, protein-DNA, and genetic interactions, pathways, genes physiological and biochemical translation, gene and protein expression, protein domain, and phenotypic screening (34).

Results

Consensus modules and genes associated with wound healing

The presence of potential outliers may impact subsequent analyses and lead to potentially misleading findings. To address this concern, hierarchical cluster analysis was employed to identify and exclude outlier samples. From the clustering tree analysis, two outliers, identified as GSM6348 and GSM6349; were detected and subsequently

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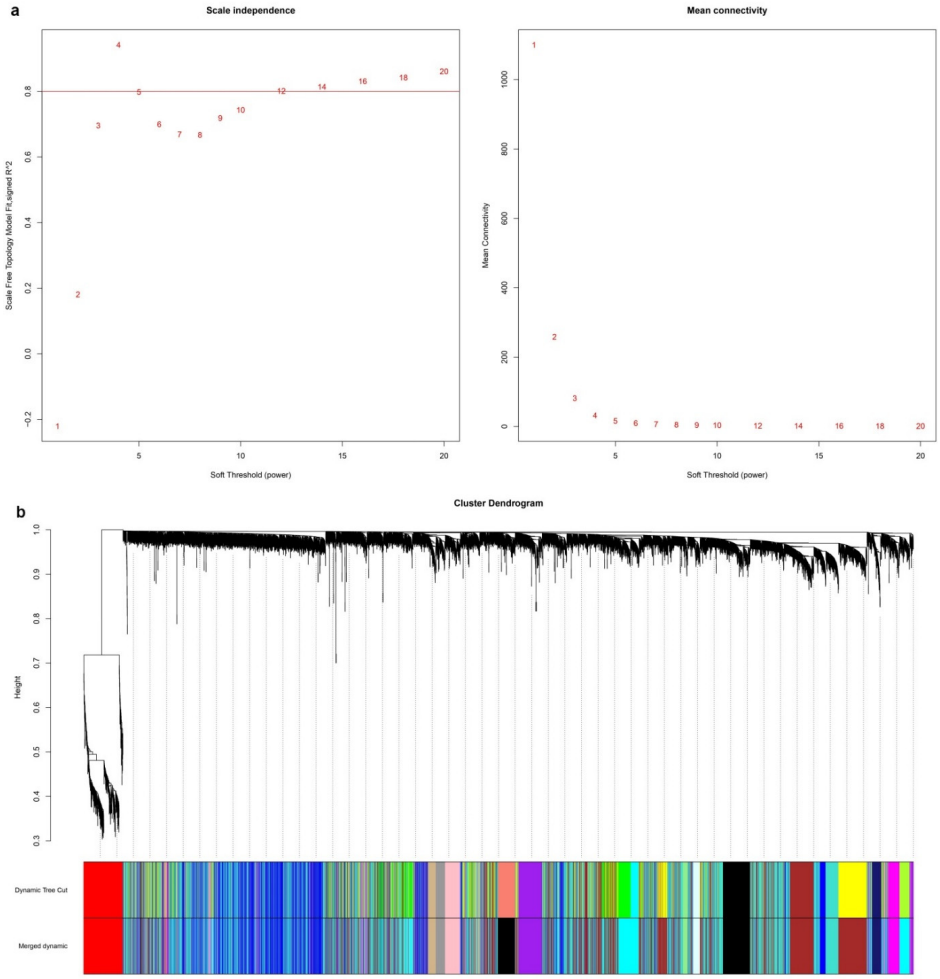

Figure 1. a. Scale independence and mean connectivity analysis related to wound healing. The proper soft threshold power = 12 was selected. *b.* Clustering dendrogram of wound healing-related modules, with dissimilarity based on the topological overlap.

removed. Following outlier removal, the clustering tree was re-plotted to confirm the absence of outliers. Utilizing criteria aimed at minimizing connection measurements, a soft-thresholding power of 12 was determined as suitable for each dataset in this study (Figure 1a). Subsequently, a total of 14 consensus gene co-expression modules were generated through combination and analysis (Figure 1b).

Relating consensus modules to wound healing and functional annotation

Clinical trait heatmap and sample dendrogram showed in Figure 2a. The tables of module-trait relationships elucidated the connections between the clinical traits (wound healing and control in Figure 2b) and the consensus modules in each data set. Notably, two relationship tables demonstrated a level of similarity. Specifically, the black ($r = -0.41$, $P = 0.02$), brown ($r = -0.41$, $P = 0.02$), and light-green modules ($r = -0.41$, $P = 0.02$) exhibited significant associations with clinical traits across both matrices. These modules included 475, 1710, and 69 genes, respectively. Subsequently, a functional enrichment analysis was performed on genes co-expressed within these modules to unveil their potential role in wound healing.

Pathway analysis from KEGG revealed specific pathways associated with each module. The brown module was linked to the Mitogen-Activated Protein Kinase (MAPK) signaling pathway, apoptosis, ErbB signaling pathway, and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance (Figure 3a). In contrast, the black module was associated with mitophagy-animal, FoxO signaling pathway, shigellosis, NOD-like receptor signaling pathway, and autophagy-animal (Figure 3b). Lastly,the light-green module was connected to Herpes simplex virus 1 infection, Epstein-Barr virus infection, Human cytomegalovirus infection, Human immunodeficiency virus 1 infection, Kaposi sarcoma-associated herpesvirus infection, Osteoclast differentiation, and Antigen processing and presentation (Figure 3c).

Modules hub genes related to wound healing phenotypes and functional interaction network

Based on our findings above, a total of 16 genes were identified as potential hub genes. Out of these, 13 genes belong to the brown module, while the remaining three genes are associated with the black module. Specifically, the hub genes identified within the brown module include

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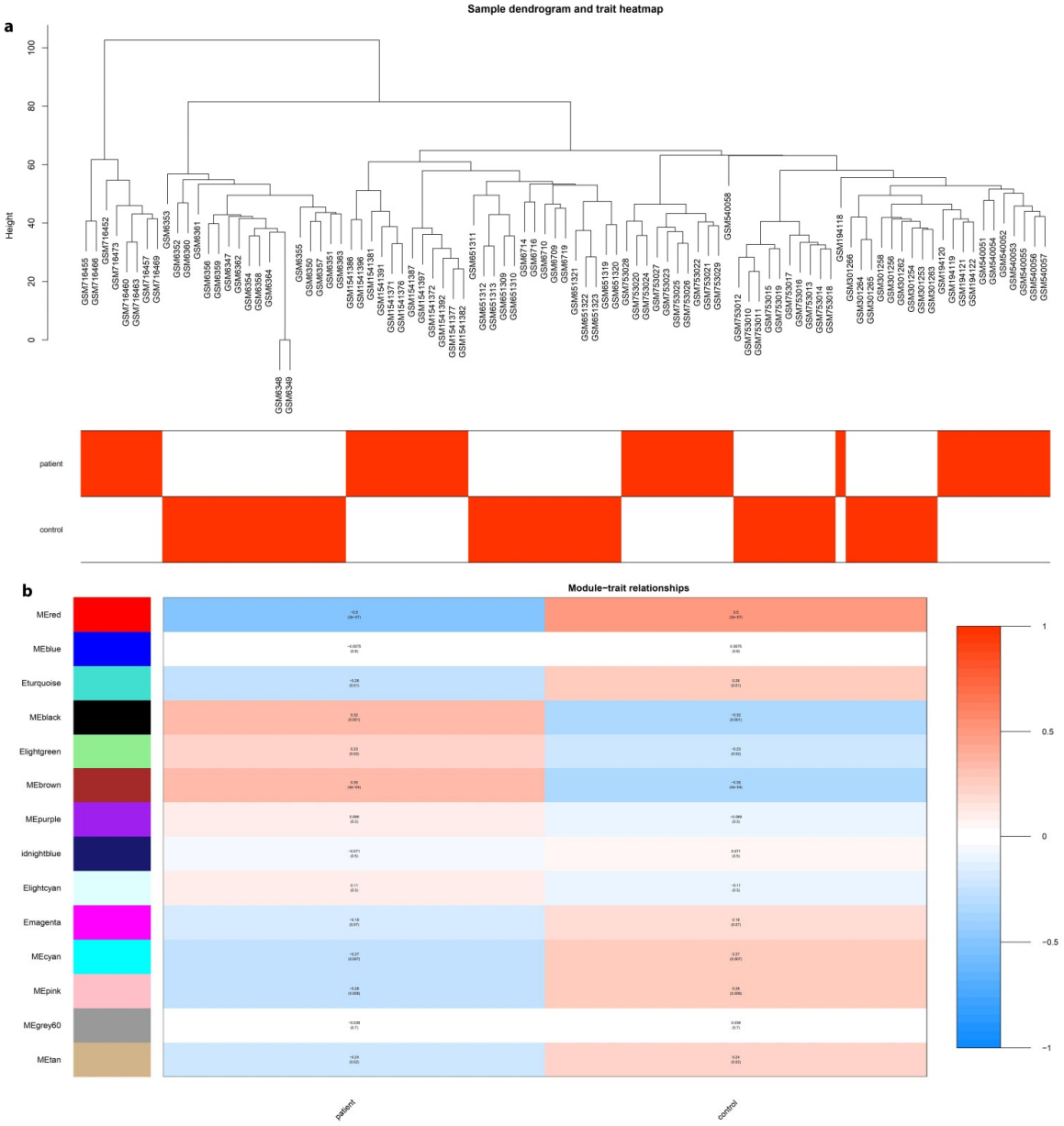

Figure 2. a. Clinical trait heatmap and sample dendrogram. The sample dendrogram's threshold value is set at 100 to remove samples with considerable variability. *b.* Module-trait relationship heatmap. Hierarchical clustering of module eigengenes that represent the clustering analysis's modules. The module is represented by the row, while the column represents the trait. The p-values in the box show the correlation and p-value.

CDK2AP2, PPP1R15A, SEC61A1, ISG20, RHOG, NFKBIE, PLAUR, MAP2K1, BYSL, VCP, ZYX, IL1RL1, JOSD1, while the hub genes in the black module were *TAB2, YTHDF3, and PRKAR1A*. Figure 4 illustrates the gene-gene functional interaction network for these module hub genes related to wound healing phenotypes, comprising 36 nodes and 104 edges.

Discussion

Wound healing is a normal response to tissue damage and involves several overlapping phases (35). Numerous cellular components and structures play a role in the dynamic process of wound healing.

These cellular and molecular activities are meticulously coordinated and regulated. Changes to the actin cytoskeleton, as well as the production of extracellular matrix (ECM) proteins and integrin receptors, are required for efficient tissue healing and restoration of tissue function (36). Consequently, it is essential to examine the hub genes' critical pathways related to wound healing for the diagnosis and treatment of individuals with wound healing dysfunction.

The weighted gene co-expression network analysis method has been utilized to extract valuable information from a gene expression microarray profile by constructing

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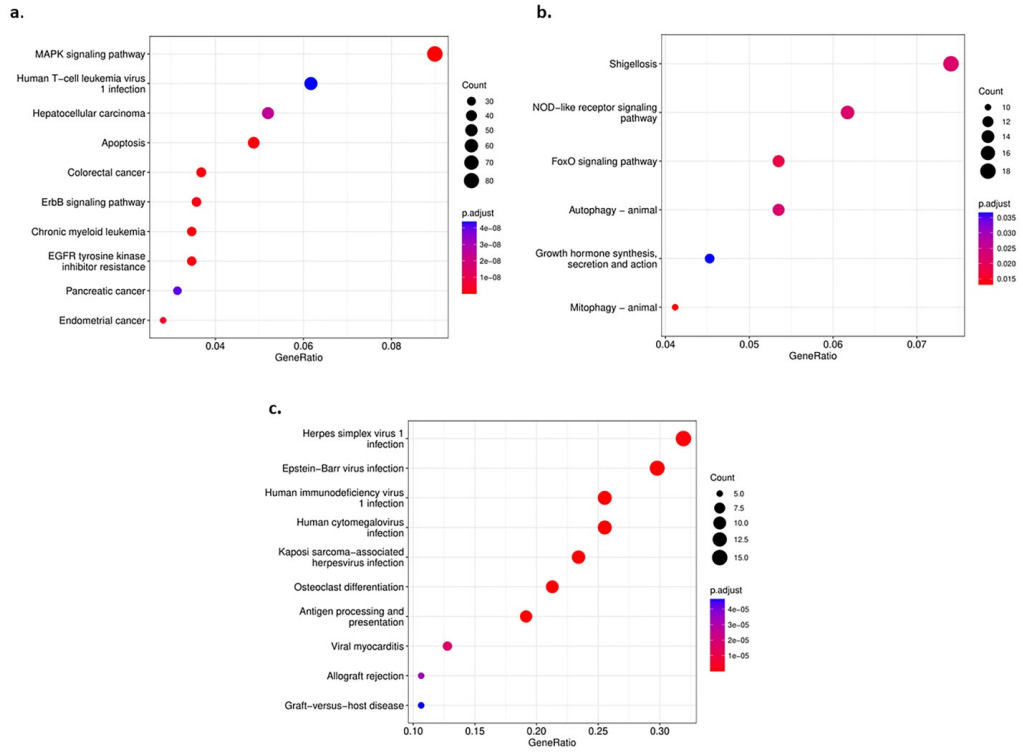

Figure 3. Bubble chart of KEGG pathway analysis for desired modules. *a.* Brown module KEGG pathway analysis. *b.* Black module KEGG pathway analysis. *c.* Light-green module KEGG pathway analysis.

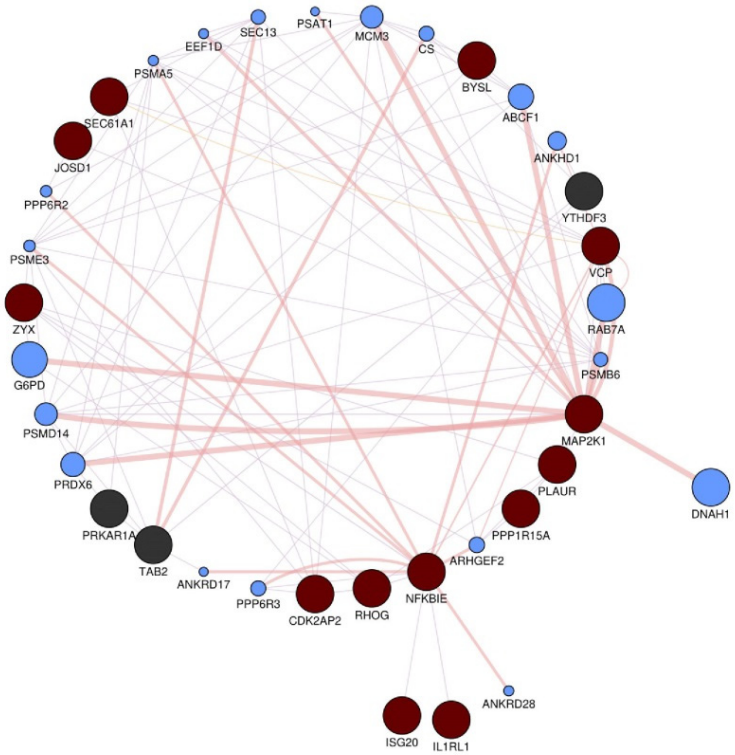

Figure 4. The gene-gene functional interaction network. Brown circles represent hub genes related to the brown module, and black circles represent hub genes related to the black module.

gene modules and explaining the importance of a gene module from a biological perspective. This research discovered that the brown, black, and light-green modules were highly correlated with wound healing traits. There were 475, 1710, and 69 genes in the black, brown, and light-green modules, respectively. In addition, 16 genes in

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the brown and black modules were identified as hub genes. These hub genes may have significant clinical implications in diagnosing and prognosis of wound-healing diseases.

The Kyoto Encyclopedia of Genes and Genome enrichment analysis has revealed that brown module co-expressed genes are mainly enriched in the signaling pathways, including MAPK, EGFR, ErbB, and apoptosis. MAPK signaling plays a crucial role in vital cellular functions like growth, differentiation, and migration. The MAPK families exhibit remarkable conservation and comprise three kinases: C-Jun N-terminal kinase/stress-activated protein kinase, classical MAPK (ERK), and p38 kinase (37). The MAPK/ERK signaling pathway in cells has been extensively researched in relation to cell proliferation and migration, which are triggered by cellular inducers like growth factors.Furthermore, activating the MAPK/ERK signaling pathway is considered a potential treatment for wound healing (38-41). Consequently, growth factors and cytokines activate this pathway, leading to downstream responses. In cellular mitogenesis, this process is known as a cellular checkpoint (37). Further investigation is warranted to explore the involvement of cyclins, a protein family responsible for regulating cell cycle progression through the activation of cyclin-dependent kinases (42), in the cyclin pathway within the context of MAPK signaling during wound healing.EGF receptor, also known as EGFR, ErbB-1, or HER1 in humans, is predominantly found in epithelial cells and plays a crucial role in promoting cellular proliferation, migration, and survival (43). When EGFR interacts with its primary ligand EGF, EGFR signaling occurs, which may induce angiogenesis (44, 45). Angiogenesis is crucial for wound healing as it enables oxygen and nutrients to enter wound sites (46). EGFR signaling is implicated in various biological processes, including angiogenesis, by regulating endothelial cell proliferation, death, and migration (47). Previous research has shown that elevated glucose levels have the potential to interfere with EGFR signaling and impede the process of corneal epithelial wound healing (48). A study in a pig model of wounds has demonstrated that transfecting EGFR promotes wound resurfacing when paired with epiregulin or heparin-binding EGF (49). Furthermore, apoptosis plays a role in regulating cell proliferation and eliminating cell populations that have completed their respective activities without causing tissue injury or an inflammatory response throughout the various stages of wound healing (50, 51).

The signaling pathways associated with KEGG enrichment of the black module include the FoxO signaling pathway and the NOD-like receptor signaling pathway, the components of which have been examined in wound healing-related research. $FOXO_3$ gene performs a critical role in the development of radiation-induced skin fibrosis by participating in the P53 pathway and engaging in basic cellular processes like apoptosis, cell survival, and cell cycle regulation (52, 53). The pathophysiology of various inflammatory skin disorders is also associated with the Nod-like receptor protein (NLRP)-3 inflammasome/IL-1 pathway (54). In this regard, Weinheimer-Haus et al. discovered that NLRP-3 null mice and caspase-1 null animals exhibited delayed re-epithelialization, granulation tissue development, and angiogenesis compared to WT mice, demonstrating the importance of NLRP-3 signaling in the early stages in wound healing (54). Research on signaling pathways enriched in brown and black module genes suggests the significance of these pathways in wound healing traits and indicates that they may be the primary pathways for further study in wound healing research.

KEGG enrichments of light-green module remarkably identified that the co-expressed genes are significantly enriched in virus infection pathways, including Herpes simplex virus 1, Epstein-Barr, Human cytomegalovirus (HCMV), Human immunodeficiency virus 1 (HIV1), and Kaposi sarcoma-associated herpesvirus. Hedner et al. reported that primary herpes simplex virus (type 1) infection delays the healing of oral excisional and extraction wounds in rats, whereas antiviral therapy with acyclovir (ACV) decreases inflammation and improves the healing of infected wounds (55). Furthermore, immunocompromised HIV-AIDS patients experience impaired wound healing and an increased risk of wound infection (56). HIV-positive patients without AIDS seem to have an increased risk of wound complications, but those with AIDS are more likely to experience prolonged wound healing (56, 57). In addition, Dumortier et al. discovered that the HCMV secretome includes various components that contribute to wound healing, such as growth factors, ECM and ECM-modifying proteins, and the ELR including CXC chemokines, cytokines, enzymes, and adhesion molecules (58). It should be highlighted that numerous viral infections may probably impair wound healing through the involved pathways, although further research in this area is necessary.

Other pathways prominent in the black module's KEGG analysis include autophagy and mitophagy. Autophagy plays a crucial role in regulating wound healing throughout the stages of hemostasis, inflammation, proliferation, and remodeling. It enhances the durability, proliferation, and migration of various cell types, such as neutrophils, macrophages, endothelial cells, keratinocytes, and fibroblasts. This, in turn, facilitates their biological activities and contributes to the promotion of wound healing (59). Mitophagy (mitochondrial-specific autophagy) is the autophagic process responsible for eliminating damaged or overloaded mitochondria (60). Mitophagy suppresses cell apoptosis by eliminating damaged or dysfunctional mitochondria. Malfunctioning mitochondria can negatively impact the cell through the production of high levels of reactive oxygen species (ROS) and the release of pro-apoptotic signals such as cytochrome c (61). Notably, modifications in mitophagy occur in rats with burn wounds (62). Sirt3 specifically modulates mitophagy to improve diabetic corneal epithelial wound healing in vivo and in vitro (63). Mitophagy, like autophagy, has been investigated rarely in wound healing. The findings of this study suggest that autophagy and mitophagy may have a role in wound healing-related traits, which has to be validated in future research.

On the other hand, the hub genes were recognized, and some of these genes have been studied for their role in wound healing. Some of these hub genes have potential roles in wound healing and wound healing traits. PLAUR produces the receptor for urokinase plasminogen activator

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and, given its association in localizing and expanding plasmin production, without a doubt controls various ordinary and neurotic forms connected to cell-surface plasminogen enactment and bounded extracellular matrix breakdown (64). Notably, the expression of *PLAUR* was elevated in diabetic mouse corneas in response to injury, and this overexpression was dramatically inhibited by hyperglycemia (65). Furthermore, Diaz et al. discovered that UPA-mediated crosstalk between N-cadherin and β- catenin advances wound healing, as doe's β-catenin-triggered transcription of the uPA receptor (PLAUR), which is considered necessary for uPA to actuate astrocytic wound healing (66). *PLAUR* is an ECM- remodeling gene that is highly expressed in radiation wounds (67).

Following ER stress resolution, the eIF2 dephosphorylation guided by *PPPIR15A*/*GADD34* reverses the inhibition of general protein translation (68). Protein phosphatase 1 regulatory subunit 15A (*PPP1R15A*) expression levels were significantly decreased in Idiopathic pulmonary fibrosis (69). Furthermore, in a rabbit ear wound model, Leung et al. found that *PPP1R15A* was a 30-fold increase in Pseudomonas aeruginosa infected wounds compared to uninfected wounds (70). *PPP1R15A* may have a function in wound healing, although its expression levels need to be studied further. Furthermore, RHOG which is Ras Homology Growth-related or (ARGH) is a compact (21 kDa) monomeric G protein or GTP-binding protein that plays an essential role in numerous intracellular signaling pathways (71). According to Bass et al., RHOG gene disruption in mice delays cutaneous wound healing owing to a migratory deficit in RHOG null fibroblasts and keratinocytes (72).

Zyxin (*ZYX*) is a phosphoprotein in human cells with an apparent molecular weight of 84 kDa (73). In fibroblasts, ZYX and integrin are found together in areas where the cells attach to the substratum, known as focal adhesions. ZYX is evenly distributed along actin-containing stress fibers and epithelial circumferential actin bundles. The highest concentrations of ZYX are observed at the ends of stress fibers or at sites of focal cell adhesion (74). The amount of *ZYX* and its relative shift in the location of keratinocytes moving to wounds imply that it functions in structuring the cytoskeleton of actin bundles (75). Sabino et al. (76) discovered the relevance of zyxin during wound healing, which is consistent with prior in vitro studies (75) have emphasized the involvement of zyxin in the efficient healing of scratched cell monolayers, as evidenced by the delayed scratch closure observed upon zyxin suppression in MDCK cells (77). Sec61α1 was likewise one of the genes in which de novo mutations induced a wound-healing deficiency in this child with severe congenital neutropenia (78). Overall, although the other hub genes identified in this study, which include *CDK2AP2, ISG20, NFKBIE, MAP2K1, BYSL, VCP, IL1RL1, JOSD1, TAB2, YTHDF3,* and *PRKAR1A*, have not been studied in wound healing studies, this study confirms these genes as targets with potential for further wound healing studies.

The key benefit of this research is that it combines wound healing patients' gene expression data with clinical traits. The WGCNA algorithm may create the gene network following the scale-free network distribution and partitioning genes with comparable expression into the same module by selecting the suitable weighting coefficient to weight the correlation coefficient between genes. The modules with the highest connectivity attributes were then chosen, and hub genes within the modules were discovered. Finally, gene enrichment analysis investigated the possible link between modules and hub genes.

Limitation

Our research has the following limitations: First, we confirmed our results using data from a publicly accessible database and did not conduct further research to assess the expression of associated genes or the biological processes and pathways involved. Second, since our research relies on data from a shared database and internet technology, we cannot guarantee the accuracy of our results. Finally, our studied data was almost exclusively restricted to a single location and did not demonstrate this problem on a global scale, despite our efforts to cover this by using a vast number of datasets. Further well-designed biological research with high sample numbers is required to validate our results.

Conclusion

Our results identified the related consensus modules of nine wound healing microarray datasets. Enrichment analysis using KEGG was conducted for the co-expression genes within these modules. and the pathways that were most associated with the development of wound healing traits, such as autophagy and mitophagy, and cell signaling pathways such as MAPK, EGFR, and ErbB2, were discussed. The hub genes obtained in this study are among the genes that each have the potential to be the basis for future studies. These results cast a minnow to catch a whale and lay the foundation for future studies into the implications of these co-expressed modules on wound healing traits.

Authors' Contributions

All the authors made contributions to the conception and design of the study. M.F and F.M were responsible for material preparation, data collection, and analysis. The initial draft of the manuscript was authored by M.F. A.S and F.M provided feedback on earlier versions of the manuscript. The final manuscript was reviewed and approved by all authors.

Ethical Considerations

This study received approval from the ethics committee at Iran University of Medical Sciences under the ethics code 'IR.IUMS.REC.1398.03'.

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Conflict of Interests

Ali Samadikuchaksaraei serves as a shareholder and CEO at Baztarmim Company, a company primarily dedicated to manufacturing tissue engineering products. The other authors do not have any competing interests to disclose.

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