Integrated Bioinformatic Analysis of Differentially Expressed Genes Associated with Wound Healing

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Abstract

Objective: Wound healing is a complex process involving the coordinated interaction of various genes and molecular pathways. The study aimed to uncover novel therapeutic targets, biomarkers and candidate genes for drug development to improve successful wound repair interventions.

Materials and Methods: This study is a network-meta analysis study. Nine wound healing microarray datasets obtained from the Gene Expression Omnibus (GEO) database were used for this study. Differentially expressed genes (DEGs) were described using the Limma package and shared genes were used as input for weighted gene co-expression network analysis. The Gene Ontology analysis was performed using the EnrichR web server, and construction of a protein-protein interaction (PPI) network was achieved by the STRING and Cytoscape.

Results: A total of 424 DEGs were determined. A co-expression network was constructed using 7692 shared genes between nine data sets, resulting in the identification of seven modules. Among these modules, those with the top 20 genes of up and down-regulation were selected. The top down-regulated genes, including *TJP1, SEC61A1, PLEK, ATP5B, PDIA6, PIK3R1, SRGN, SDC2,* and *RBBP7*, and the top up-regulated genes including *RPS27A, EEF1A1, HNRNPA1, CTNNB1, POLR2A, CFL1, CSNk1E, HSPD1, FN1*, and *AURKB*, which can potentially serve as therapeutic targets were identified. The KEGG pathway analysis found that the majority of the genes are enriched in the "Wnt signaling pathway".

Conclusion: In our study of nine wound healing microarray datasets, we identified DEGs and co-expressed modules using WGCNA. These genes are involved in important cellular processes such as transcription, translation, and post-translational modifications. We found nine down-regulated genes and ten up-regulated genes, which could serve as potential therapeutic targets for further experimental validation. Targeting pathways related to protein synthesis and cell adhesion and migration may enhance wound healing, but additional experimental validation is needed to confirm the effectiveness and safety of targeted interventions.

Keywords: Bioinformatics, Gene, Network-Meta Analysis, Regeneration, Wound Healing

Introduction

Wounds are a common occurrence in everyday life and can result from a variety of causes, including trauma, surgery, and chronic conditions such as diabetes. The restoration of tissue integrity and function is an essential process of wound healing, and involves a complex interplay of genes, proteins, and molecular pathways (1). Understanding its molecular mechanisms may develop effective therapeutic interventions, while promoting a successful tissue repair.

Previous studies have demonstrated the potential of using omics data from non-wound healing patients to gain insights into the molecular mechanisms in wound healing and related diseases. By identifying key genes and genetic variants associated with chronic wounds, researchers can gain a better understanding of the underlying biological processes and potentially develop more effective treatments and therapies for these conditions (2). To gain a better understanding of wound healing processes and ultimately to achieve better methods of wound management and treatment, we need newer and better qualified network-based methods besides laboratory data analysis based on statistical tests.

By doing this study, we can view wounds as distressed molecular networks which arrange the tools for exploring and enhancing the healing process. It leads to improved consideration of the process complication such as (A) the molecular pathways linked to wound healing, (B) the

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pathway interactions through various stages of wound healing, and also (C) the probability of holding the mechanism of regulatory interactions of wound healing.

Material and Methods

Gene Expression Omnibus datasets

This study is a network-meta analytical study. In this study, we included expression microarray datasets listed in the Gene Expression Omnibus (GEO) of the NCBI official website (https://www.ncbi.nlm.nih.gov/geo/) by the end of December 31, 2021. To find datasets reporting the data on expression levels of mRNAs in the wound healing, the following search keywords were used: human wound healing, omics, transcriptomic, genomic, wound repair, regeneration, wound repair, and genetic variation. This search strategy retrieved 7500 datasets. Our inclusion criteria were human subjects and high throughput microarray data. Further, the exclusion criteria were included: i. Samples which had any accompanying disease, ii. Subjects who received any treatments for wound healing, iii. Data derived from a cell line, and iv. Data derived from non-blood samples.

These datasets were manually screened for having data on skin and oral wound healing of human subjects. Manual screening only short-listed 65 datasets. These 65 datasets underwent quality control and were checked against a set of inclusion and exclusion criteria.

Finally, only 9 datasets were selected and included in the present study, which, in total, contained data from 73 patients and 42 healthy controls (a sum of 115 human subjects). The microarray datasets were all obtained from the GPL570, GPL96, and GPL8300 platforms of the GEO

with the origin of skin and oral wounds. Table 1 provides the details of the datasets included in this study.

Data were analyzed using the Robust Rank Aggregation (RRA) method (|logFC [fold-change] | >1.5 and an adjusted P<0.001). Rank analysis was used to determine the total number of differentially expressed genes (DEGs). The expression data in our study were quality corrected and quantile normalized using the Affy package implemented in the R programming language (version 4.0.2) (3). The datasets were integrated at mRNA levels using the random effect method (REM) and then up- and down-regulated expressed genes were identified by the MetaDE package in R (version 2.2.1) (4). A principal component analysis was performed on the merged data for normalization in order to exclude heterogeneous data. The MetaQC tool in R (version 0.1.13) (5) was used to assess the studies' quality and consistency.

Data preprocessing and differential expression analysis

Initially, the preprocessing step for raw data were used the Robust MultiArray Averaging (RMA) method in the Oligo package in R (version 1.38.0) (6). According to the Platform annotation data, the probes, that lacked similar gene symbols were deleted. The gene expression value was calculated using the average value of the probes that were mapped to the same gene symbol. Genes with a |LogFC|>1.5 and an adjusted P<0.001 were identified as DEGs using the Linear Models for Microarray Data (Limma) tool in R (7)`zR#. The input data for weighted gene co-expression network analysis (WGCNA) were shared genes from nine datasets (8).

Table 1: Characteristics of selected datasets

No.	Wound healing categories	GEO ID	Platform	Number of subjects	Subjects' group
1	Skin and oral	GSE21648	Affymetrix, GPL96	15	Patients
2	Skin	GSE30355	Affymetrix, GPL570	10	Patients
3	Skin	GSE7890	Affymetrix, GPL570	10	Patients (with no HT)
4	Oral	GSE28914	Affymetrix, GPL570	8	Patients
5	Skin	GSE63107	Affymetrix, GPL570	30	Patients
6	Skin	GSE11919	Affymetrix, GPL570	9	Normal subjects
7	Skin	GSE440	Affymetrix, GPL8300	5	Normal subjects
8	Skin	GSE26487	Affymetrix, GPL8300	10	Normal subjects
9	Skin	GSE427	Affymetrix, GPL8300	18	Normal subjects

GEO; Gene Expression Omnibus, ID; Identification, and HT; Hydrocortisone treatment.

Construction of co-expression modules of datasets by WGCNA

The WGCNA using the WGCNA package in R was undertaken to assess the relative significance expression of genes and their module memberships. Co-expression networks were established in our study using a soft threshold power in order to provide different modules with different expression patterns. The Pearson correlation coefficient was then used to analyze the weighted co-expression connections in the adjacency matrix. The matrix was transformed into the Topological Overlap Matrix (TOM) using a similar function, and then used to assess the co-expression associations between genes. The networks were constructed by grouping numerous genes with comparable co-expression patterns. Consequently, the list of modules related to up- and down-regulated genes and their co-expressed genes were selected.

The protein-protein interaction network

The Search Tool for the Retrieval of Interacting Genes (STRING) version 11.5 (http://string-db.org/) (9). The web server was used to acquire the protein-protein interaction (PPI) network. The Cytoscape was used to visualize the PPI network (v3.7.2; https://cytoscape.org (10); The Cytoscape Consortium, San Diego, CA). This phase included a list of the top-ranked up and down-regulated genes and all desired modular genes.

Functional Annotation of differentially expressed genes and desired modules

The EnrichR, an interactive and collaborative *HTML5* gene list enrichment analysis tool, *(*https://maayanlab. cloud/Enrichr/) (2) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) function enrichment analysis on DEGs. We also used linear regression analysis and the Limma package in R software to compare patients' group with the group of normal subjects. After performing this analysis and using Lmfit analysis (Linear Models for Microarray Data) to fit the model in the data for each gene, we obtained an estimate of the regression coefficient of each gene, which was performed using both of eBayes command (11) and Limma package in R software (version 3.18). In the linear regression model, we calculated the correlation coefficient by the concept of $|logFC|>1.5$ to compare each gene in the two groups, and the $P<0.001$ was regarded as statistically significant.

Results

Identification of differentially expressed genes and selection of the top up and down-regulated genes

The final selected data of patients and healthy controls from GPL96 (GSE21648), GPL570 (GSE30355, GSE7890, GSE28914, GSE63107, GSE11919), and GPL8300 (GSE440, GSE26487, GSE427) platforms were entered into this study. In this data, 300 up-regulated and 124 down-regulated genes were identified (Supplementary 1 and 2, See Supplementary Online Information at www. celljournal.org).

The expressions of these genes were significantly different ($|logFC| >1.5$ and adjusted $P<0.001$) between the patient group and healthy control group. Subsequently, we selected 9 down-regulated and 11 up-regulated genes. Down-regulated genes included *TJP1, SEC61A1, PLEK, ATP5B, PDIA6, PIK3R1, SRGN, SDC2,* and *RBBP7.* While, up-regulated genes comprised *RPS27A, EEF1A1, HNRNPA1, RAN, POLR2A, CTNNB1, CFL1, CSNk1E, HSPD1, FN1,* and *AURKB.* To assess data distribution following normalization, box plots of gene expression data were depicted (Fig.S1, See Supplementary Online Information at www.celljournal.org). Separate arrays in the box plots exhibited similar medians of expression level, showing that the adjustment was performed correctly. The Figure S1 (See Supplementary Online Information at www.celljournal.org) illustrated box plots of selected gene expression data before and after normalization.

Table 2: Details of co-expressed modules with top up- and down-regulated genes

Co-expressed module	Up or down-regulation	DEGs
Turquoise	Down-regulation	*TJP1*
	Down-regulation	*SEC61A1*
	Down-regulation	*PLEK*
	Down-regulation	*ATP5B*
	Down-regulation	*PDIA6*
	Down-regulation	*PIK3R1*
	Down-regulation	*SDC2*
	Down-regulation	*RBBp7*
	Up-regulation	*POLR2A*
	Up-regulation	*CFL1*
	Up-regulation	*CSNk1E*
	Up-regulation	*AURKB*
Blue	Up-regulation	*HNRNPA1*
	Up-regulation	*CTNNB1*
	Up-regulation	*HSPD1*
	Up-regulation	*FN1*
Brown	Up-regulation	*RPS27A*
	Up-regulation	*EEF1A1*
Yellow	Down-regulation	*SRGN*

DEGs; Differentially expressed genes.

Weighted gene co-expression network analysis

In this study, a co-expression network was constructed using 7692 shared genes between nine datasets, and the WGCNA package was used to construct co-expression modules. The scale independence and mean connectivity of modules were shown in Figure 1A, B. Generating a TOM, seven modules were identified (Fig.1C). The desired modules were identified according to the merged modules.

Identification of desired modules and establishing the protein-protein interaction network

Among the seven resulting modules described above, co-expressed modules of the 20 top genes of up and down-regulation (|logFC| >1.5 and adjusted P<0.001)

were identified as desired modules (Table 2). Using the STRING database, the predicted PPI networks of desired modules were created (Fig.2). A PPI network of top up- and down-regulated genes is shown in Figure 3.

Gene Expression and KEGG enrichment analysis of desired modules

The targeted modules were considerably enriched in common GO biological processes, cellular compartments, and molecular functions, according to the GO functional enrichment analysis (P<0.05). The KEGG pathway analysis revealed that most of the enriched genes are in the "Wnt signaling pathway".
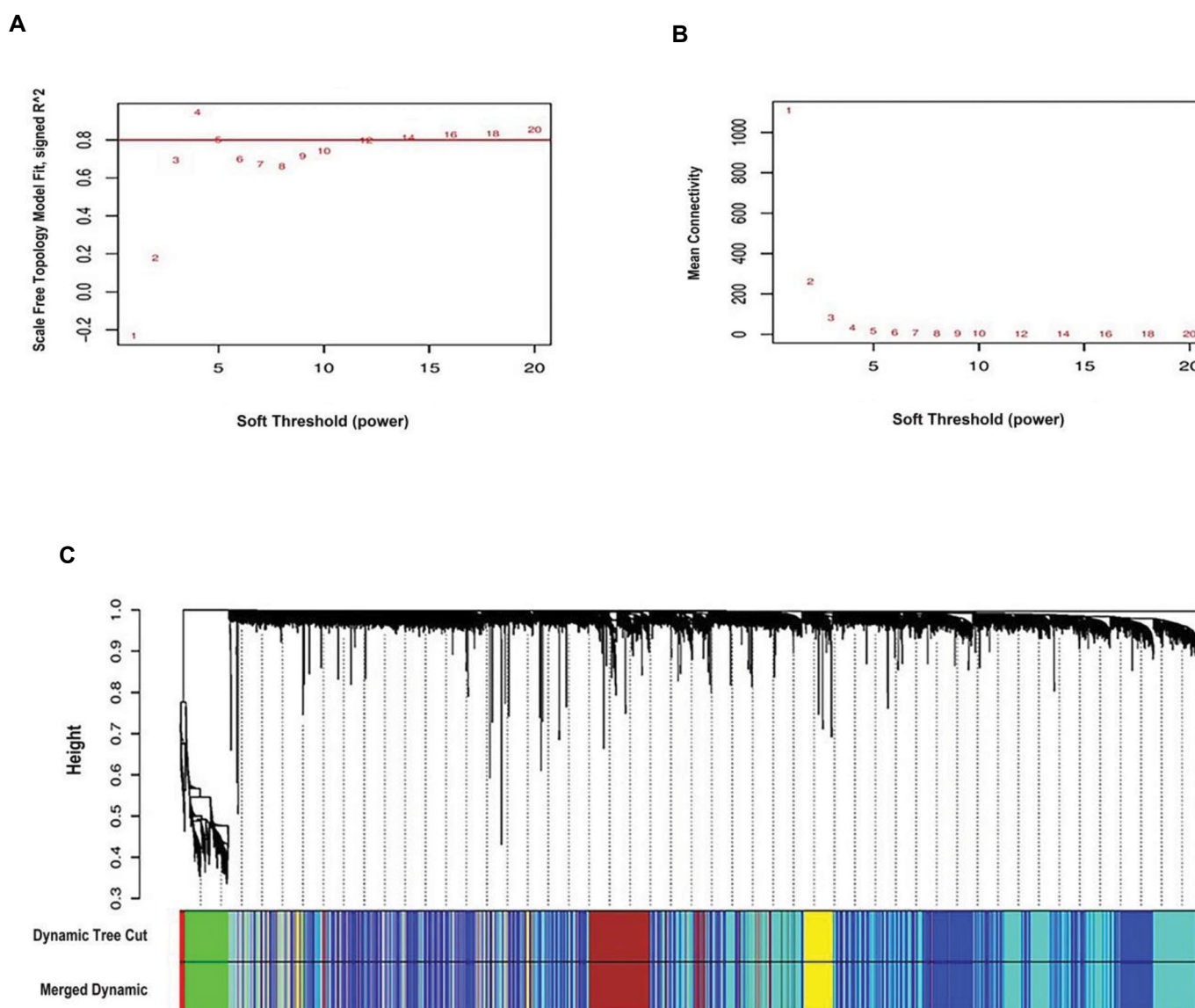

Fig.1: Soft-thresholding power and gene co-expression modules. **A.** WGCNA scale-free fit index analysis for various soft-thresholding powers (β). The appropriate soft-thresholding power=12 was chosen. **B.** Mean connectivity analysis of various soft-thresholding powers. **C.** Using average hierarchical linkage clustering to identify gene co-expression groups. The y-axis represents co-expression distance, and the x-axis represents genes. In the horizontal bar immediately below the dendrogram, modules are represented by different colors, with gray referring to unassigned genes.
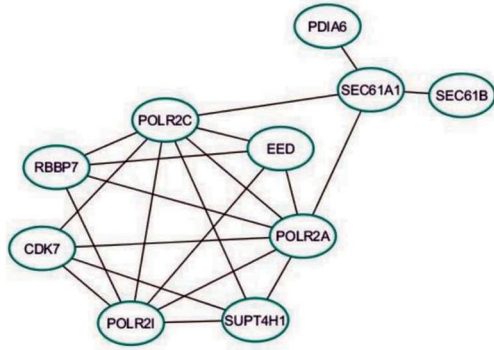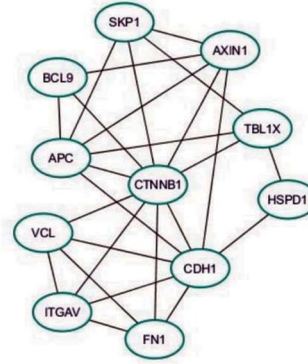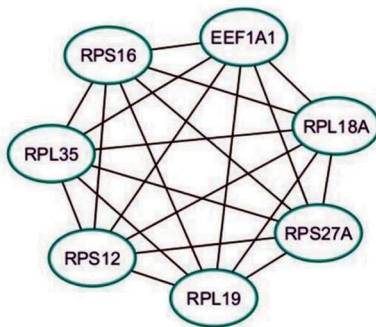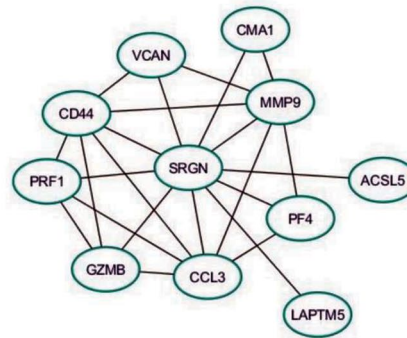
Fig.2: PPI Network. DEGs are presented in each module connecting with lines, which represent interacting proteins. Circles and lines represent genes and the interaction of proteins, respectively. PPI; Protein-protein interaction and DEGs; Differentially expressed genes.

Fig.3: PPI network of top genes. Up-regulated and down-regulated genes are illustrated in yellow and blue colors, respectively. PPI; Protein-protein interaction.

KEGG enrichment analysis

Wnt signaling pathway
Hippo signaling pathway
Focal adhesion
Proteoglycans in cancer
regulation of actin cytoskeleton

GO enrichment analysis

GO biological process

Regulation of cellular protein localization (GO:1903827)
Positive regulation of telomere maintenance via telomerase (GO:0032212)
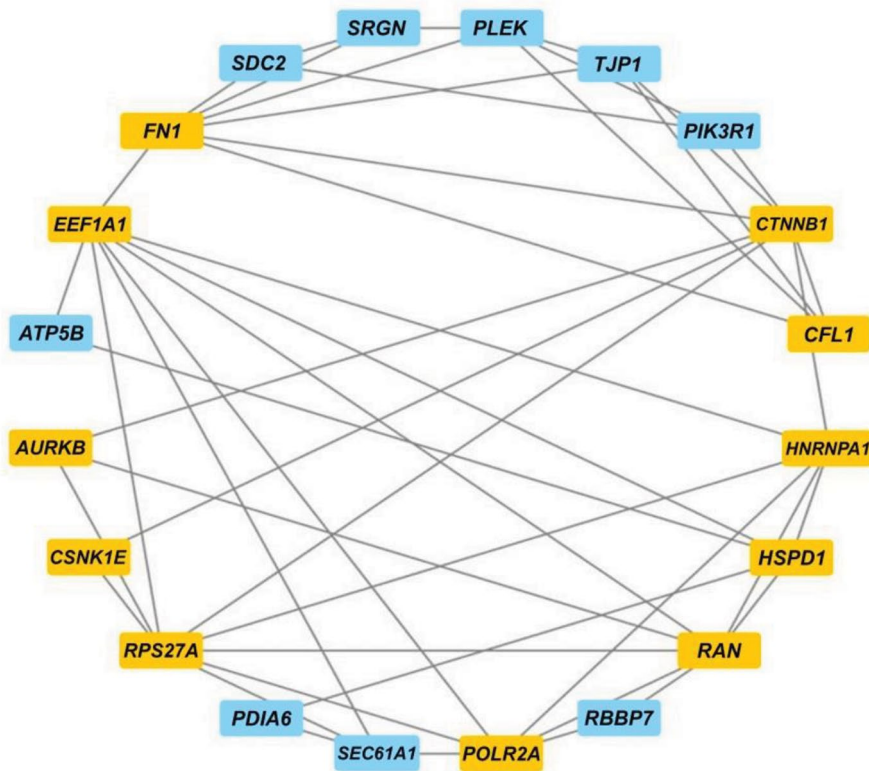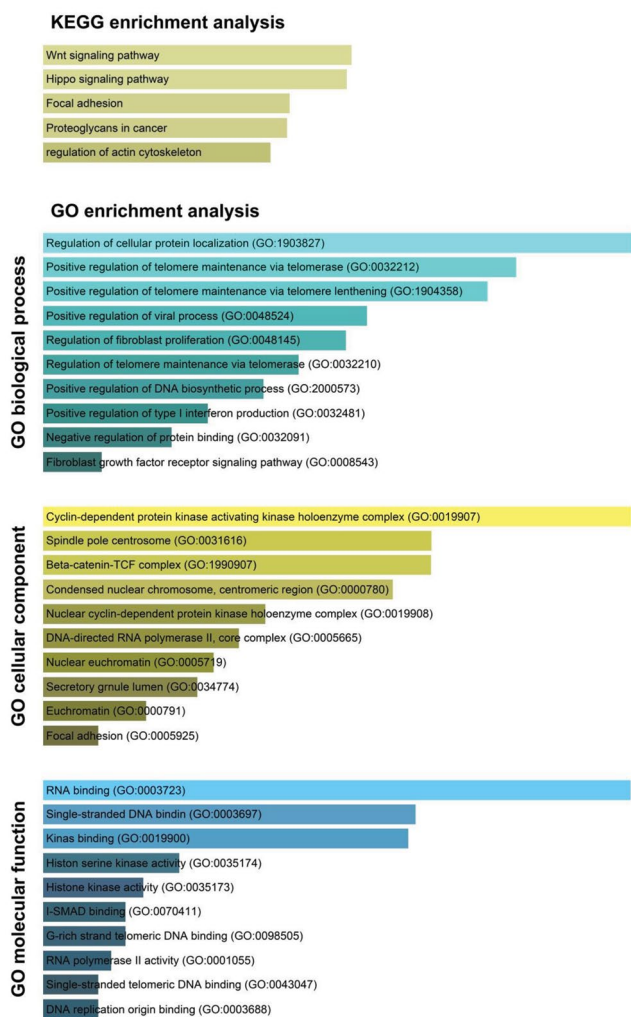Positive regulation of telomere maintenance via telomere lenthening (GO:1904358)
Positive regulation of viral process (GO:0048524)
Regulation of fibroblast proliferation (GO:0048145)
Regulation of telomere maintenance via telomerase (GO:0032210)
Positive regulation of DNA biosynthetic process (GO:2000573)
Positive regulation of type I interferon production (GO:0032481)
Negative regulation of protein binding (GO:0032091)
Fibroblast growth factor receptor signaling pathway (GO:0008543)

GO cellular component

Cyclin-dependent protein kinase activating kinase holoenzyme complex (GO:0019907)
Spindle pole centrosome (GO:0031616)
Beta-catenin-TCF complex (GO:1990907)
Condensed nuclear chromosome, centromeric region (GO:0000780)
Nuclear cyclin-dependent protein kinase holoenzyme complex (GO:0019908)
DNA-directed RNA polymerase II, core complex (GO:0005665)
Nuclear euchromatin (GO:0005719)
Secretory grnule lumen (GO:0034774)
Euchromatin (GO:0000791)
Focal adhesion (GO:0005925)

GO molecular function

RNA binding (GO:0003723)
Single-stranded DNA bindin (GO:0003697)
Kinas binding (GO:0019900)
Histon serine kinase activity (GO:0035174)
Histone kinase activity (GO:0035173)
I-SMAD binding (GO:0070411)
G-rich strand telomeric DNA binding (GO:0098505)
RNA polymerase II activity (GO:0001055)
Single-stranded telomeric DNA binding (GO:0043047)
DNA replication origin binding (GO:0003688)

Fig.4: GO and KEGG findings of the DEGs. The length of each bar shows the level of importance in that category, as determined by the p value. Also, the intensity of color of the bars shows the strength of association with that category. Lower color intensity shows stronger association. GO; Gene Ontology, KEGG; Kyoto Encyclopedia of Genes and Genomes, and DEGs; Differentially expressed genes.

The four desired modules including turquoise, blue, brown and yellow modules were identified for DEGs, resulting from our GO analysis and KEGG findings (Supplementary 3 and 4, See Supplementary Online Information at www.celljournal.org).

Discussion

An acute wound healing is a dynamic process that ultimately leads to the scar development. It seems that there are several signaling pathways and components involved in this process. One of these pathways is the β-catenin-dependent Wnt signaling, which becomes more active after a skin injury and the operation of exogenous Wnt3a (rmWnt3a). Wnt3a promotes the maturation of the wound matrix, re-epithelialization, and the formation of a scar (12). In keratinocytes and fibroblasts, Catenin beta 1(β-catenin) that coding by *CTNNB1* has different effects, as it restricts the migration of keratinocytes while encouraging the proliferation of fibroblasts. This suggests that β-catenin may either impede or improve the healing process (13).

Likewise, the *FN1* gene is present at all phases of wound healing. The *FN1* gene provides instructions for making two types of the fibronectin-1 proteins, including soluble plasma fibronectin-1 (pFN1) and insoluble cellular fibronectin-1 (cFN). The pFN1 mainly functions in the early stages of wound healing to aid in forming clots. Platelets, via increased platelet binding site expression, aid in assembling plasma-derived *FN1* into fibrillar matrices. Through locally expressed *FN1* assembly, cellular-derived *FN1* governs the latter phases of a tissue remodeling. The FN1 protein is linked to the collagen III matrices during the granulation stage; the temporary FN1 matrix is then reconstructed into the collagen I matrix, which is surrounded by FN1 (14).

The Cofilin-1 (CFL1) is an actin-remodeling protein that binds to G- and F-actins and induces a pH-sensitive depolymerizing activity that regulates cell motility during tissue repair (15). Both form of the CFL1 protein, including phosphorylated and dephosphorylated, were also detected in platelets, with the latter quantity corresponding to the later phases of platelet aggregation in wound healing, that indicated a newly dephosphorylated CFL1, might play an essential role in the cytoskeletal remodeling that happens during platelet aggregation, a critical step in the early stages of wound healing (16).

Many top-up-regulated genes are strongly associated with one of the four stages of wound healing processes, but in other conditions such as cancer, they are also involved in proliferation. For example, *RPS27a* (ribosomal protein S27a) plays a role in ribosome synthesis and protein post-translational modifications. The *RPS27a* gene has been discovered to have a significant role in a cell proliferation through proliferation stimulation, cell cycle progression control, and apoptosis suppression in leukemia cells (17). The *POLR2A* gene, which encodes the RNA polymerase II subunit A, is strongly associated with cancer development. Studies have demonstrated that *POLR2A* enhances the expression of cyclin and cyclin-dependent kinases (CDKs) at different stages of gastric cancer, suggesting its involvement in promoting cell cycle progression (18). Further studies are required to establish conclusive evidence and its role in wound healing.

In addition, knocking down the *eEF1A1* gene, eukaryotic elongation factor 1A1, has a notable impact on reducing proliferation and activation of apoptosis in Jurkat cells, which may be mediated via the PI3K/Akt/NF-B and PI3K/Akt/mTOR signaling pathways (19). Remarkably, *CSNk1E* gene has been identified as a significant contributor to activated β-catenin signaling in cancer, suggesting that it might be a viable therapeutic target for malignancies that contain an active β-catenin protein (20). The heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) which encoded by *HNRNPA1* gene, is a stress granule protein that plays an important role in the development, proliferation, and metastatic cancer cells

(21). So based on the research, there is a clear relationship between protein biogenesis and wound healing that could be a potential target for therapeutic approaches.

Furthermore, in the present study, nine genes of highest down-regulation rate were identified, including *TJP1, SEC61A1, PLEK, ATP5B, PDIA6, PIK3R1, SDC2, RBBp7,* and *SRGN.*

The *PIK3R1 gene* is one of the HIF-1α pathway-associated genes, with a greater expression rate in the skin and the tongue (22). In a next-generation sequencing (NGS) experiment, *PIK3R1* was found to be one of the down-regulated genes in irradiated mouse skin (23). Radiation-induced skin fibrosis is a typical complication of radiotherapy in the clinical practice. In this side effect, *FOXO3* plays a key role as one of the central genes in causing radiation-induced skin fibrosis. Fibrosis is linked to the regulation of the *FOXO3* gene, which plays a role in the P53 pathway and is involved in essential cellular processes such as apoptosis, cell survival, and the regulation of the cell cycle. Additionally, the PIK3R1 gene can also regulate the expression of *FOXO3* (24).

In this background, the Syndecan-2 a protein in humans is encoded by the *SDC2* gene. *SDC2,* is elevated during fibrosis alike other *SDCs*. It is produced by fibroblasts and stimulated by transforming growth factor β1 (TGFβ1) and insulin-like growth factor binding protein-3 (25). The *SCD2* is among the up-regulated genes in the axolotl wound healing process, along with *FN1, TGFβ1,* and *LTBP3*. It is implicated as a mediator in the TGFβ-signaling pathway (26). Similarly, in *SCD2* knock-down mice, the loss of this protein resulted in impaired retinal artery development and vascular branching. In addition, the wound healing was problematic in these mice (27).

Likewise, research has demonstrated that lower gene expression in *SRGN* leads to decreased proliferation and impaired wound healing ability in endothelial cells (28). Also, in a study of a patient with severe congenital neutropenia with the de novo *SEC61A1* mutation, which was reported for the first time, problematic wound healing was addressed as one of the symptoms causes (29). Studies of inflammatory genes and inflammatory processes in wound healing have shown that *PLEK* is one of the central inflammatory genes and acts as a signal transducer in the migration of polymorphonuclear neutrophilic granulocytes to skin lesions which are classified as up-regulated genes in this process (30).

The Tjp (ZO-1) protein, plays a crucial role in wound healing by regulating the assembly and disassembly of tight junctions, as well as promoting cell migration and re-epithelialization (31). In a study that examined expression of genes at 48 hours, 72 hours, and five days after the wound healing process, the *ATP5*B gene, an expression reference gene, showed a minor stability in comparison with other twelve genes (32). The Retinoblastoma Binding Protein 7 (RBBP7), may potentially engage in the early phases of wound healing by serving as a chromatin remodeling factor, which requests additional investigation (33).

Lastly, because of the effects of genes that have not been studied in the wound healing process, including *TJP1, SEC61A1, PLEK, ATP5B, PDIA6, PIK3R1, SRGN, SDC2, RBBp7, RPS27A, EEF1A1, HNRNPA1, POLR2A, CFL1, CSNk1E, HSPD1, FN1,* and *AURKB,* play a crucial role in the cell proliferation by contributing to essential aspects of the cell cycle or the regulatory pathways of the process. While, a cell proliferation refers to the growth and division of cells, it is necessary for the development, maintenance and repair of tissues and organs in the body. Although, additional research is needed, these genes probably play crucial and influential roles in wound healing, with an elevated expression but fine-tuned regulation.

Analyzing these datasets with R software revealed 424 DEGs composed of 300 up-regulated and 124 down-regulated genes between wound samples and normal skin. Several studies have shown that the Wnt gene family can affect wound healing. Wounding can activate Wnt signaling genes in several levels of wound healing. Moreover, Wnt signaling genes are responsible for different stages of skin formation, and this feature makes it a good target for targeting in tissue regeneration studies (34).

Our GO analysis of DEGs demonstrated that a "regulation of cellular protein localization" is one of the mechanisms in wound healing. A targeted localization is a type of regulation that is common in eukaryotes. It is possible for cells to achieve rapid changes in the function of local proteins by particularly redirecting the distribution of a number of existing proteins. Eukaryotic cells have developed sophisticated targeting mechanisms to ensure that proteins are delivered to the correct cellular location. Indeed, essential stages such as the proliferation phase, the supply of protein sources is a priority of wound healing, and any disruption in this process will lead to disruption of it. The enrichment of DEGs in terms such as "positive regulation of telomere maintenance via telomerase" and "positive regulation of telomere maintenance via the telomere lengthening" suggests that any process that stimulates or increases the frequency and extent of the addition of telomeric repeats by telomerase is crucial for effective wound healing. In this aspect, mice with dangerously short telomeres exhibit difficulties with highly proliferative tissue, such as poor wound healing, inflammatory skin lesions, early hair loss, and early hair graying (35). GO and KEGG analyses of up- and down-regulated gene expression showed that the wound healing mechanism is a multidimensional process that comprises multiple signaling pathways and important molecular mechanisms.

Bioinformatics analysis indicated that these genes and pathways could be involved in wound healing processes in several ways. According to GO analysis, the main enrichments of turquoise module related to biological processes include "positive regulation of transcription, DNA-templated", "positive regulation of transcription by RNA polymerase II", "cellular protein modification

process", "mRNA processing" and "positive regulation of nucleic acid-templated transcription" each of which, except for the cellular protein modification process, is somehow related to the transcription process. It is also interesting to note that the blue module enrichment analysis, which is also co-expressed with the majority of up-regulated genes, showed a high overlap in the enrichment of these modular genes with the turquoise module enrichment analysis closely related to the transcription process. During wound healing, several transcription factors coordinately regulate target genes, which vary over time (36), enabling the integration of external stimuli to provide the necessary physiological response. The capacity of numerous transcription factors to bind to these regulatory regions in distinct cell types or respond to stimuli is frequently determined by pre-existing genome-wide chromatin accessibility.

Interestingly, the enrichment analysis of brown module genes suggests that these genes are involved in aspects of the translation process. The most significant pathway related to this module includes "SRP-dependent co-translational protein targeting to membrane". The targeting of proteins to membranes during translation is dependent on two key elements, including the signal-recognition particle (SRP) and the SRP receptor. The SRP is a cytosolic particle that transitory binds to the endoplasmic reticulum signal sequence, the large ribosomal unit, and the SRP receptor in the ER membrane. However, the role of SRPs in the wound healing procedure is closely related to *CELF1,* an RNA-binding protein gene, that is responsible for regulating SRPs. When *CELF1* is depleted in myoblast cells, the wound healing process is disrupted by altering the regulation of SRPs. Comparable abnormalities in a wound healing are found when an SRP subunit imbalance is created by an over-expression of SRP68, which may be a limiting factor in the assembly of functional SRP (37).

After the transcription and translation process, the enrichment of the cellular protein modification process introduces a precise concept. This concept denotes the covalent modification of one or more amino acids in proteins, peptides, and nascent polypeptides (co-translational and post-translational modifications) occurring at the individual cell level. This process is essential in developing tissue specificity for wound healing (38). On the other hand, this protein modification is one of the processes that affect inflammation, which is one of the four main stages of the wound healing process. The post-translational modification (PTM) of the constituents of the inflammatory pathway, for instance Toll-like receptors (TLR) pathways, RIG-I-like receptor (RLR) pathways, NOD-like receptor (NLR) pathways, intracellular DNA sensors, intracellular RNA sensors, and inflammasomes, is critical in the control of these signaling pathways (39). The primary types of a PTM include ubiquitination, phosphorylation, polyubiquitination, methylation, and acetylation, and also, they each serve a particular function in signaling control. A PTM effects a range from the production of pro-inflammatory molecules to the interaction of adapters and receptors, as well as cell translocation in response to infectious or other damaging agents. One of the main problems disrupting the wound healing process is chronic inflammation. Meanwhile, studies have been conducted on other complications that have been dedicated to down-regulated genes and their effects on inflammatory processes and state that down-regulated genes can affect inflammation (40).

Conclusion

In our analysis of nine wound healing microarray datasets, we identified up- and down-regulated genes and co-expressed modules using WGCNA. DEGs play a role in crucial cellular processes such as transcription, translation, and post-translational modifications. Specifically, we found nine down-regulated genes, including *TJP1, SEC61A1, PLEK, ATP5B, PDIA6, PIK3R1, SRGN, SDC2,* and *RBBp7,* as well as ten up-regulated genes: *RPS27A, EEF1A1, HNRNPA1, CTNNB1, POLR2A, CFL1, CSNk1E, HSPD1, FN1,* and *AURKB.* These discoveries offer valuable insights and potential therapeutic targets for further experimental validation. Targeting pathways related to protein synthesis may enhance cellular activity during wound healing, while interventions aimed at promoting proper cell adhesion and migration could facilitate efficient wound closure. However, additional experimental validation is necessary to confirm the efficacy and safety of any targeted interventions.

Acknowledgements

Authors' Contributions

M.F.; Methodology, Data validation, Formal analysis, Investigation, Data curation, Original draft writing, Review, Editing, and Visualization. F.M.F.; Methodology, Data validation, Formal analysis, Review, Editing. N.S.; Methodology, Data validation, Formal analysis, and Data curation. A.S.; Conceptualization, Project supervision, Project administration, and Funding acquisition. All authors read and approved the final manuscript.

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