Gene Co-expression Network Analysis for Identifying Modules and Functionally Enriched Pathways in Vitiligo Disease: A Systems Biology Study

Afshin Derakhshani[1], Homa Mollaei[2], Negin Parsamanesh[3], Mohammad Fereidouni[4], Ebrahim Miri-Moghaddam[5], Saeed Nasseri[4], Yongwen Luo[6], Hossein Safarpour[4], and Behzad Baradaran[1,7]

[1] *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[2] *Department of Biology, Faculty of Sciences, University of Birjand, Birjand, Iran*
[3] *Zanjan Metabolic Diseases Research Center, Zanjan University of Medical Sciences, Zanjan, Iran*
[4] *Cellular and Molecular Research center, Birjand University of Medical Sciences, Birjand, Iran*
[5] *Department of Molecular Medicine, Cardiovascular Diseases Research Center, School of Medicine,*
Birjand University of Medical Sciences, Birjand, Iran
[6] *Department of Biological Repositories, Zhongnan Hospital of Wuhan University, Wuhan, China*
[7] *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

ABSTRACT

Vitiligo is the most common cause of skin, hair, and oral depigmentation which is known as an autoimmune disorder. Genetic and environmental factors have important roles in the progression of the disease. Dysregulation of gene expression, like microRNAs (miRNA), may serve as major relevant factors. Several biological processes are involved in vitiligo disease and developing a comprehensive approach helps us to better understand the molecular mechanisms of disease.

In this research, we describe how a weighted gene co-expression network analysis as a systems biology approach assists to define the primary gene modules, hub genes, and messenger RNA (mRNA)-miRNA regulatory network in vitiligo disease as the novel biomarkers.

The results demonstrated a module with a high correlation with vitiligo state. Moreover, gene enrichment analysis showed that this module's genes were mostly involved in some biological activities including G protein-coupled receptors signaling pathway, lymphocyte chemotaxis, chemokine activity, neutrophil migration, granulocyte chemotaxis, etc. The co-expression network was constructed using top hub genes of the correlated module which are named as *CXCL10, ARL9, AKR1B10, COX7B, RPL26, SPA17, NDUFAF2, RPF2, DAPL1, RPL34, CWC15, NDUFB3, RPL26L1, ACOT13, HSPB11,* and *NSA2.* MicroRNAs prediction tool (miRWalk) revealed top miRNAs correlated with the interested module. Finally, a drug-target network was constructed which indicated interactions of some food and drug administration (FDA) approved drugs with hub genes.

Our findings specified one important module and main hub genes which can be considered as novel biomarkers for vitiligo therapeutic purposes.

Keywords: MicroRNAs; Systems biology; Vitiligo

Corresponding Authors: Behzad Baradaran, PhD;
Immunology Research Center, Tabriz University of Medical
Sciences, Tabriz, Iran. Tel: (+98 413) 3371 440; Fax: (+98 413) 3371
311, E-mail: baradaranb@tbzmed.ac.ir

Hossein Safarpour, PhD;
Cellular and Molecular Research Center, Birjand University of
Medical Sciences, Birjand, Iran. Tel: (+98 56) 3238 1258;
E-mail: H.safarpour@bums.ac.ir

INTRODUCTION

Vitiligo is an acquired depigmentation skin disorder characterized by progressively melanocytes dysfunction and follows multifactorial inheritance.[1,2] The prevalence of the disease ranges from 0.4 to 2.0 and so far, there is no well-known, effective, and safe therapy for vitiligo.[3,4] The exact molecular mechanisms underlying vitiligo pathogenesis are not fully clarified yet, however, various theories suggest that genetic predisposition, as well as environmental and immunological factors, could strongly evidence for loss of the depigmentation process.[5] This lack of appropriate treatment shows that proper biomarkers are immediately needed not only for differentiating vitiligo from other depigmentation disorders, but also for predicting vitiligo progression and monitoring therapeutic responses.

In this immune disease, like other ones, the dysregulation of genes expression could serve as the main cause of the disease progression. It would be due to the distributing signaling pathways or alternating the microRNA (miRNA) expression level that might lead to the further dysregulation in the expression of related genes. miRNA are non-coding RNA molecules with a short length of ~19-25 nucleotides regulating and balancing different gene functions through the post-transcriptional stage mostly via binding to the complementary region in 3' untranslated region (3'UTR) of targeting messenger RNA (mRNA).[6,7] These non-coding RNAs are contributing to the multiple cellular and physiological processes including signal transduction, differentiation, metabolism, and immune responses in humans.[8] The identity of the non-coding RNA pathways could lead to the development of miRNA-based prediction, prognosis, and target therapy strategies for vitiligo. Several studies carried out to date indicate that miRNAs have a strong diagnostic and prognostic potential in several epidermal diseases such as psoriasis,[9] atopic dermatitides[10], and allergic contact dermatitis.[11] Nevertheless, only a few reports on the predictive potential of miRNA in vitiligo are available.[12,13] The role of miRNAs in vitiligo is a promising area of research for a better understanding of the molecular and pathological mechanisms occurring in the disease.

Nowadays, the rapid growth of high-throughput technologies (such as microarray and RNA-seq) has provided new approaches for gene expression profiling.

The systems biology approach as a holistic method is a good choice for biomarker discovery in a pool of genes involved in complex diseases. Therefore, co-expression network reconstruction from the whole transcriptome profile can highlight the critical transcripts and their relation through the same network. There are several co-expression network inference methods and among them, the weighted gene co-expression network analysis (WGCNA) algorithm is more appropriate to detect key genes related to a sample trait in a network of co-expressed genes. The most interconnected genes in a module (hub genes) are often functionally significant and can, therefore, play the main role, particularly in the case of vitiligo, and represent candidate diagnostic biomarkers or potential therapeutic targets.

Therefore, the current study aimed to (i) construct consensus modules across microarray-based mRNA expression profiles from vitiligo patients and healthy individual derived tissue samples; (ii) correlate key gene modules with the vitiligo; (iii) enrich target genes from important modules to explore the possible biological processes, pathways, and vitiligo regulatory transcriptional factors,(iv) identify miRNA-mRNA bipartite networks involved in the vitiligo pathogenesis and (v) construct a drug-target network for targeted therapy in vitiligo.

MATERIALS AND METHODS

Dataset Identification and Processing

The vitiligo microarray dataset was downloaded from the national center for biotechnology information (NCBI) gene expression omnibus (GEO) database by the accession number of GSE75819. This dataset contains a total of 30 tissue samples mRNA expression profiling of lesional (n=15) and non-lesional samples (n=15) based on the GPL6884 Platform (Illumina HumanWG-6 v3.0 expressionBeadChip).[14] Quantile normalization of raw data and subsequent data processing were performed using the R software packages (R version 3.2.0). The probe-set annotation file released by Illumina was utilized to find the official gene symbol and probe ID. Genes with the coefficients of variation<0.1 were discarded. Then the average expression info indication was performed for every sample. The study protocol was approved by the research ethics committee of Tabriz University of Medical Sciences (no:IR.TBZMED.VCR.REC.1399.151).

WGCNA and Functional Module Extraction

WGCNA is a well-established method for analysis of gene expression data and investigates network alterations. In this study, we used mRNA expression data from both lesional and non- lesional (control) groups and then reconstructed them by standard WGCNA procedure. First, Pearson's correlation matrix was computed for all pair-wise mRNAs. Next, the β (soft thresholding power beta) parameter is used to adjust the scale-free property of the networks. The power of β=5 (model fitting index R^2=0.95) was chosen under the scale-free topology criterion. Afterward, the topological overlap matrix (TOM) was calculated from the adjacency matrix to assess the interconnectedness of the mRNAs. Subsequently, the dynamic tree cut algorithm with the "*cutreeDynamic*" function was used to classify similar expression mRNAs into the gene modules with a minimum module size value 30 genes. Highly similar modules were merged upon the module eigengene (ME) distance threshold of 0.1 and each module was assigned a conventional color scheme and genes which not classified in a correlated module were grouped in the grey module. To filter the co-expression modules down to those that are significantly relevant to the vitiligo, using module-trait relationship analysis of WGCNA, we calculated the ME which is the first principal component and describes most of the variation in each module. A module was chosen for further analysis if it presented a module-trait relationship>0.9 and a *p* value <0.05.

Identifying Hub Genes and Target miRNAs

A hub is a node with numerous edges in a network and might be important to the phenotype of interest. To find hub genes, gene significance (GS, the association between individual genes with traits of interest), and quantitative module membership (MM, eigengene-based intra-modular connectivity), were calculated for the selected modules. Regarding these assumptions, the similarities of all genes were quantified in every module. Specifically, genes with both GS and MM≥0.9 are considered to be hub genes. Hub genes had a tendency to define as essential gene on the network and extremely linked with other genes in the same module.

Furthermore, a co-expression network consists of hub genes of the selected module, and their co-expressed neighbor genes were constructed by GeneMANIA (https://genemania.org). Finally, miRWalk database (http://mirwalk.umm.uni-heidelberg.de/search_genes/) was used to detect miRNAs that experimentally validated as the upstream regulators of hub genes.

Functional Annotation of the Vitiligo Correlated Co-expression Modules

To further clarification of the underlying mechanism of module genes' impact on the correlative clinical feature, the clueGO (version 2.2.5) and cluepedia Plug-in tools on Cytoscape (version 3.6.0) was used to identify and visualize the module enrichment. *p* value< 0.05 was considered statistically significant.

Identification of Candidate Regulatory Drugs

Drug repositioning is an advanced method for identifying new indications of therapy for existing drugs. The well-firmly established Drug-Gene Interaction Database (DGIDB) (http://www.dgidb.org/) was used to predict functional drugs for the hub-genes. Drugs in this database are used clinically or they are presently used in clinical trials.[15]

RESULTS

Construction and Analysis of Modules Network

In this study, the GSE75819 dataset that contained mRNA expression data of lesional and non- lesional skin samples of 30 vitiligo patients was used. Quintile normalization (Figure 1A), genes conversion, and probes annotations were performed. Outlier samples were excluded from this study (Figure 1B). A β=5 was selected as a soft-thresholding power and weighted co-expression network from lesional and control samples reconstructed (Figure 1C). The hierarchical clustering dendrogram distinguished16modules after the dynamic tree cut. The Grey module contained genes, which were not fit for any groups, and it was ignored in the following steps. Figure 1D and Table 1 demonstrated module characterization.

WGCNA Modules and Module Traits Connection

The precise relation of modules with the vitiligo disorder was determined based on the module-module association and eigengene. It was shown that four modules significantly correlated with the vitiligo trait (*p* value<0.05) and 4 of them are positively related (Figure 2A). Among these modules, turquoise (correlation=0.92, *p* value=2e-07) was considered for further evaluations (Table 1).

A

B

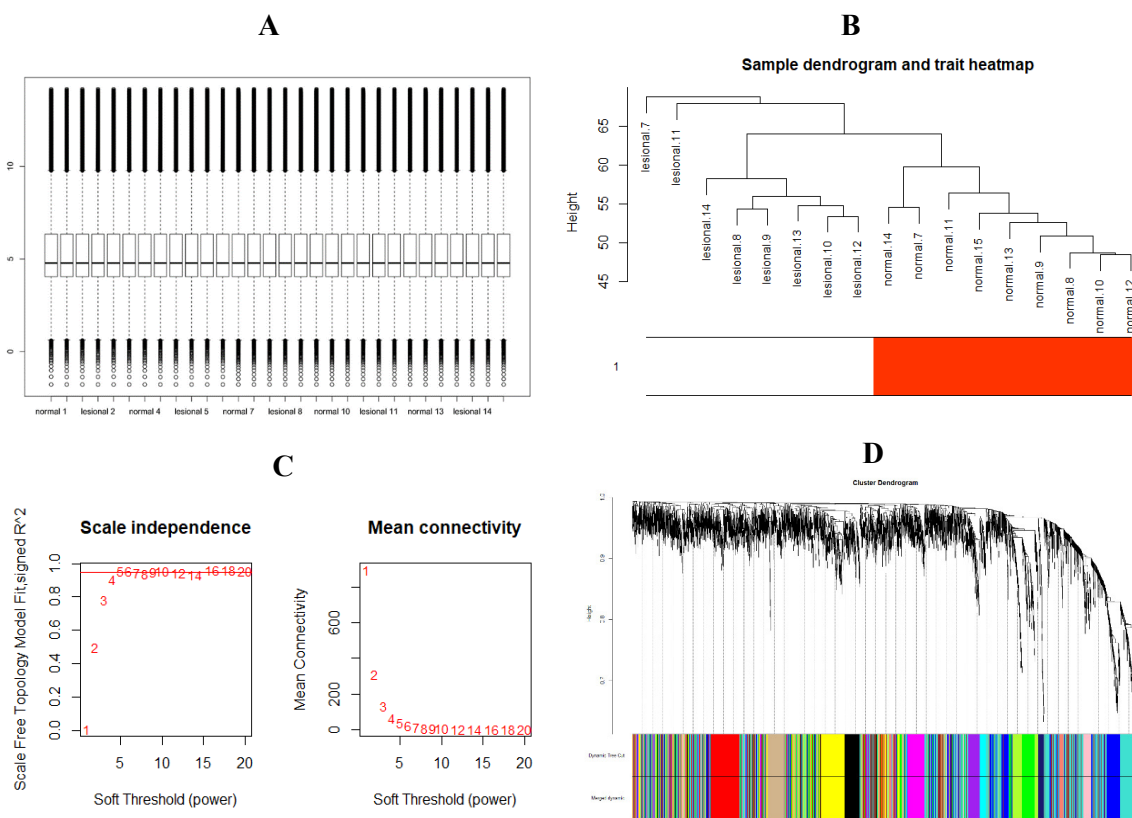Sample dendrogram and trait heatmap

C

D

Figure 1. A. Quantile-normalization of samples. B. Sample clustering to detect outliers. C. Selection of the soft-thresholding powers. D. Cluster dendrogram and module assignment from the weighted gene co-expression network analysis (WGCNA). The branches correspond to highly interconnected groups of genes. Colors in the horizontal bar represent the modules.

Table 1. Module colors characterization

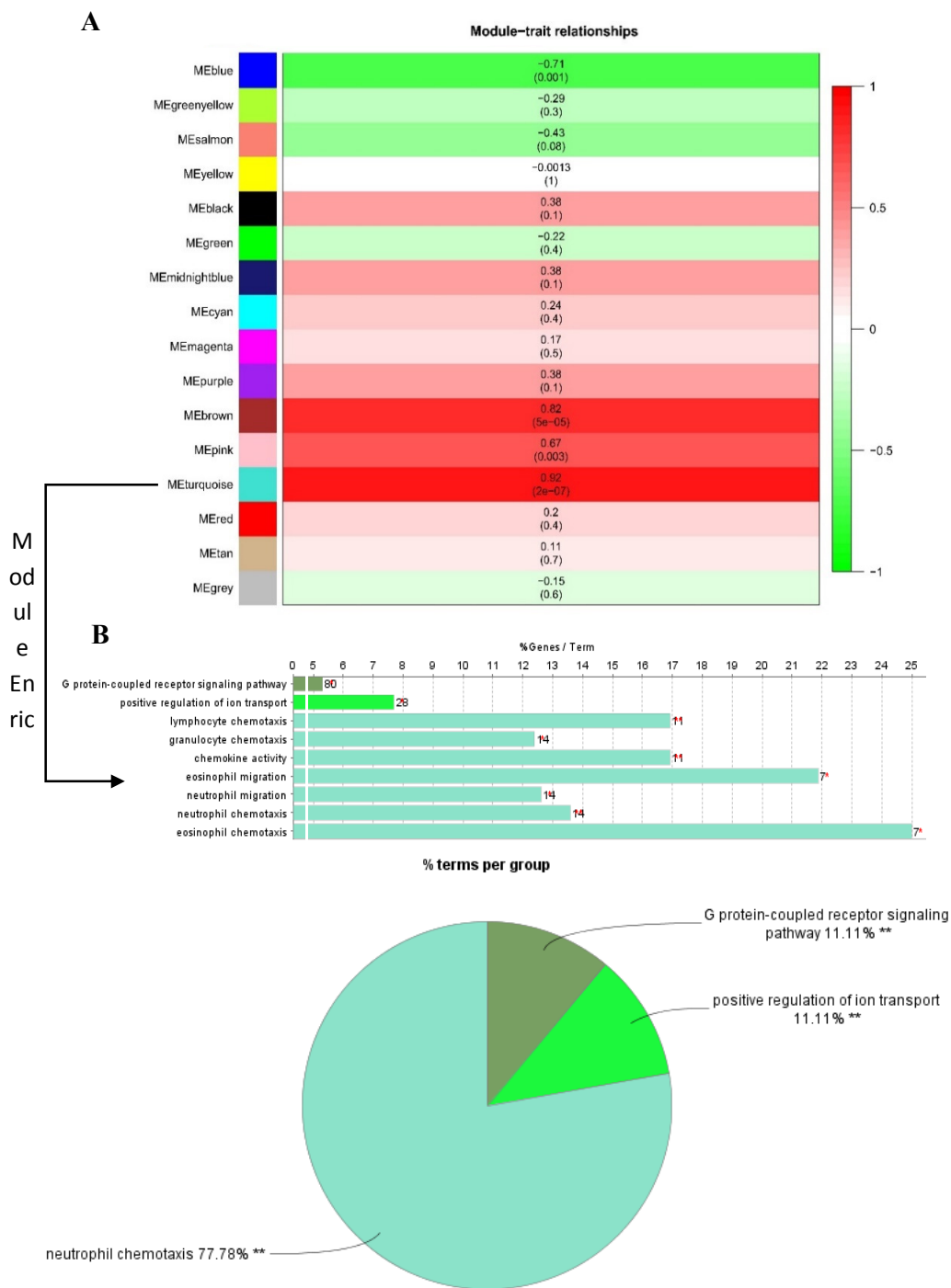| Module color	# Genes	correlation	*p* value
turquoise	773	0.92	2.00E-07
brown	398	0.82	5.00E-05
pink	179	0.67	0.003
black	186	0.38	0.1
midnight blue	84	0.38	0.1
purple	169	0.38	0.1
cyan	100	0.24	0.4
red	261	0.2	0.4
magenta	177	0.17	0.5
tan	156	0.11	0.7
yellow	374	-0.0013	1
grey	39	-0.15	0.6
green	361	-0.22	0.4
green yellow	162	-0.29	0.3
salmon	155	-0.43	0.08
blue	426	-0.71	0.001

A

B

Figure 2. A. Module-trait relationship. Each row corresponds to a module eigengene and the column corresponds to vitiligo status. The numbers in each cell represent the corresponding correlation and *p*-value. B: Functional enrichment analysis of the turquoise module.

Gene Set Enrichment Analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases preserve higher-level functional information for the systematic analysis of gene functions. In the current study, KEGG enrichment was performed for the above turquoise module to explore potential biological processes associated with the vitiligo disease. The result of KEGG pathway enrichment was showed in Figures 2B, G protein-coupled receptors signaling pathway, positive regulation of ion transport, lymphocyte chemotaxis, chemokine activity, neutrophil migration, neutrophil chemotaxis, granulocyte chemotaxis, and eosinophil chemotaxis are the top biological activities which are significantly related to the turquoise module genes and likely contribute in the vitiligo disease progression.
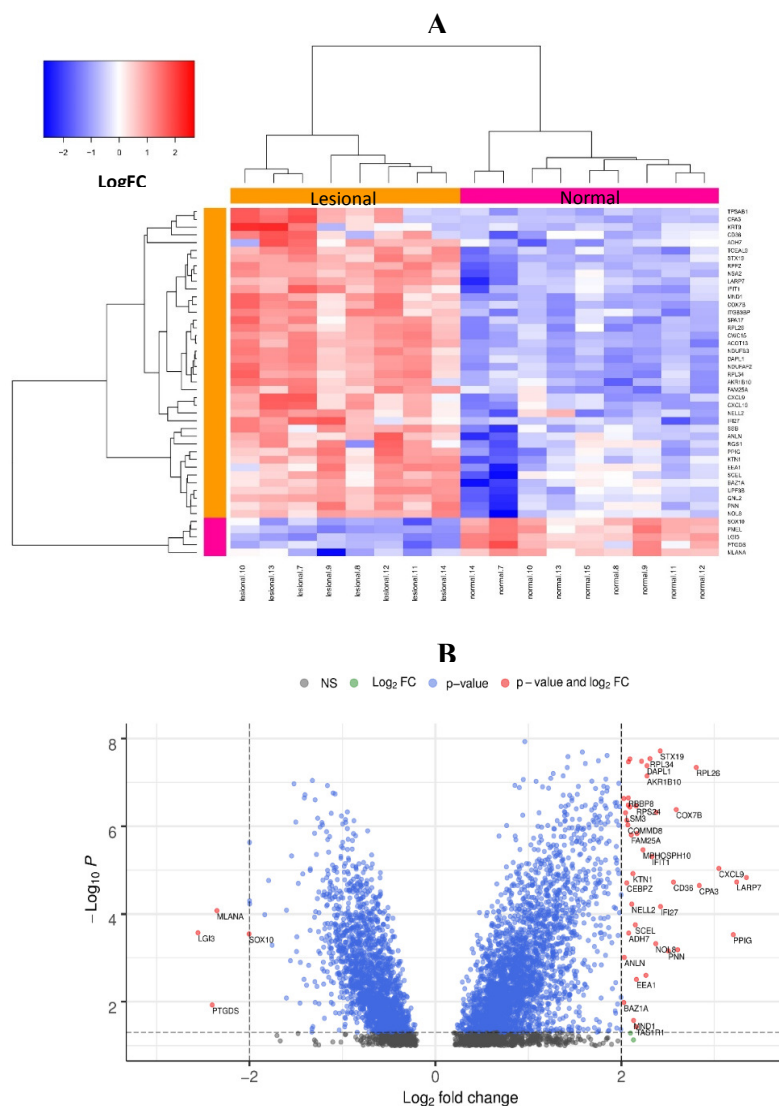

Figure 3. Hub genes detection. A. Turquoise module features of GS and MM which significantly correlated with vitiligo status (control vs. patient). Each point represents an individual gene within each module, which is plotted by GS on the y-axis and MM on the x-axis. B. Evaluation of similarity between differentially expressed genes (DEGs) and Hub genes lists; using a Venn diagram. Sixteen genes which were similar in both lists were then imported to Gene MANIA to construct a co-expression network.

Differentially Expressed Genes (DEGs) Analysis and Hub Genes Detection

With p value<0.05 and logFC$\geq$ 2, a total of 55 DEGs including 50 upregulated DEGs and 5 downregulated DEGs were identified between the lesional and non-lesional groups. The heatmap and volcano plot are shown in Figures 3A and 3B respectively. The results of the heatmap showed that these DEGs could be used to well distinguish in both lesional and non-lesional samples. Studying the correlation between features (MM and GS) (Figure 4A) of the turquoise module was led to detecting hub genes of this module that are highly associated with the vitiligo pathogenesis. They included *CXCL10, ARL9, AKR1B10, COX7B, RPL26, SPA17, NDUFAF2, RPF2, DAPL1, RPL34, CWC15, NDUFB3, RPL26L1, ACOT13, HSPB11,* and *NSA2* (Figure 4B).

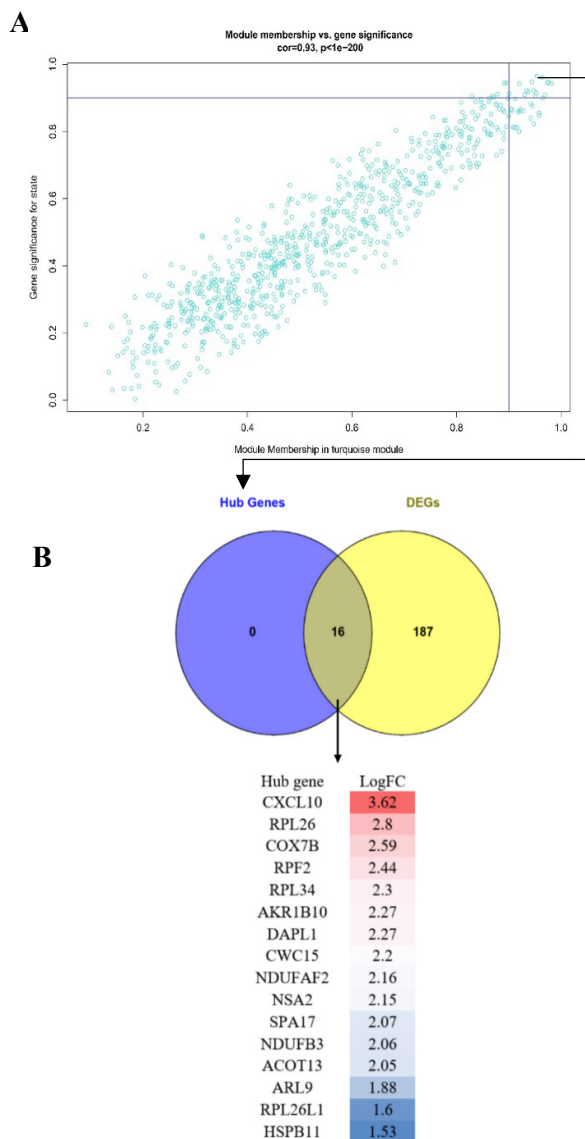| Hub gene	LogFC
CXCL10	3.62
RPL26	2.8
COX7B	2.59
RPF2	2.44
RPL34	2.3
AKR1B10	2.27
DAPL1	2.27
CWC15	2.2
NDUFAF2	2.16
NSA2	2.15
SPA17	2.07
NDUFB3	2.06
ACOT13	2.05
ARL9	1.88
RPL26L1	1.6
HSPB11	1.53

Figure 4. The heat map and volcano plot between lesional and non-lesional samples. (A) The heat map for differentially expressed genes (DEGs). (B) The volcano plot for DEGs.

Co-expression Network Construction

The co-expression network of the turquoise module has been reconstructed using GeneMANIA and Cytoscape software (Figure 5 and 6). To this end, first, we construct a co-expression network of turquoise hub genes with 4 neighbors; using GeneMANIA, and then reconstruct this network in Cytoscape.
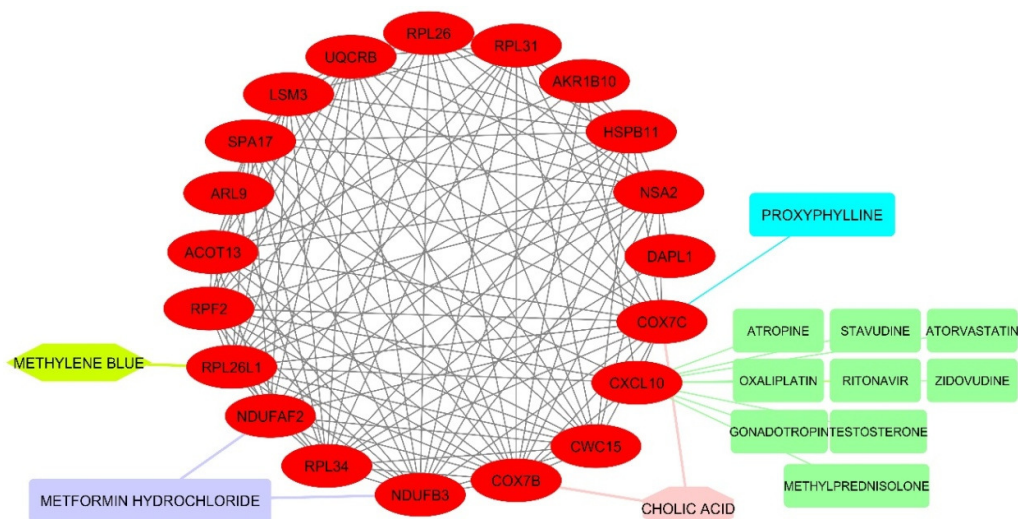

Figure 5. Drug-target network of turquoise module hub-genes: FDA approved drugs were downloaded from the drug gene interaction database (DGIdb) for each gene
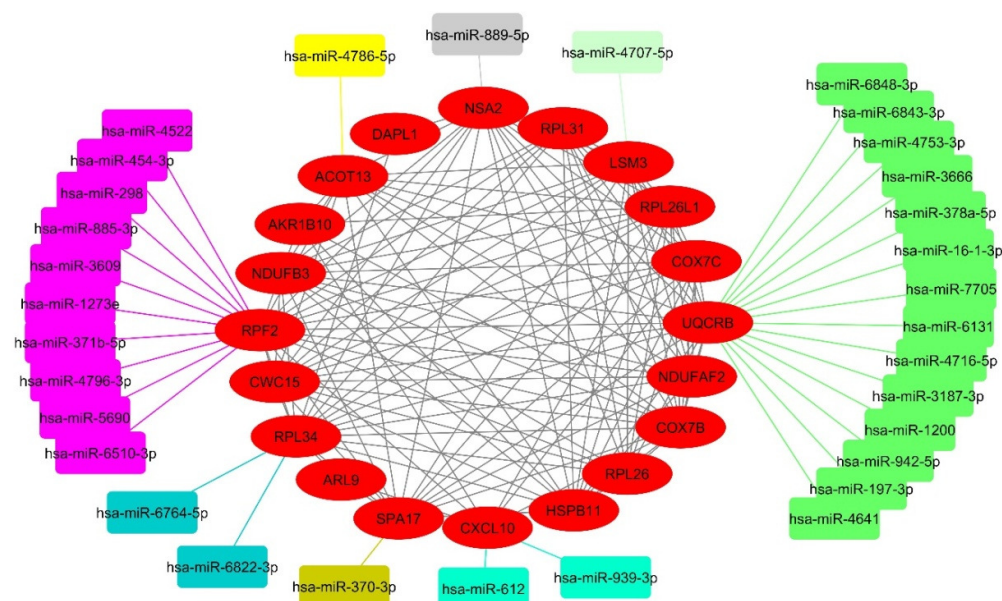

Figure 6. A miRNA-mRNA bipartite network analysis of turquoise module hub-genes: Experimentally validated miRNAs were downloaded from the miRWalk database for each gene.

Drug-target Network Construction

We also searched the module for drug targets that are presently not approved for vitiligo therapy. The proposed targets in the module are shown in Figure 5. The targets' presence in the module of interest suggests these drugs to potentially impact vitiligo and serve as the possible candidates for further research in this respect.

mRNA-miRNA Target Prediction

As co-expressed genes may be co-regulated by the common up-stream regulators like miRNAs, in the next step of our study we did gene-set enrichment analysis; using miRWalk database. The most important of miRNAs that are experimentally validated by miRTarBase filtering, as regulators of turquoise genes are visualized in Figure 6.

DISCUSSION

Vitiligo is an autoimmune disease proceeds by the damage of melanocytes and clinically distinguished by the onset of white macules and patches.[16-18] While the precise molecular mechanisms underlying vitiligo pathogenesis are not fully understood, various theories suggested that genetic predisposition, as well as environmental and immunological factors, could strongly evidence for loss of depigmentation process.[5]

WGCNA is a technique of systems biology to reveal higher-order gene interactions which can promote our understanding of the pathogenesis of vitiligo. In this study, we used this algorithm to systematically recognized the co-expressed gene modules associated with the vitiligo progression. After dataset preprocessing, module identification, gene co-expression network construction, relating modules to traits, and functional enrichment, we found that turquoise modules with 773 hub genes were associated with the occurrence of vitiligo. Module enrichment analysis demonstrated that these genes are mainly involved in some biological pathways such as G protein-coupled receptors signaling pathway, lymphocyte chemotaxis, chemokine activity, neutrophil migration, granulocyte chemotaxis. Previous studies confirmed the key roles of the chemokines and the recruitment of immune cells into tissues in autoimmune patients.[19-21]

Hub genes were including *CXCL10, ARL9, AKR1B10, COX7B, RPL26, SPA17, NDUFAF2, RPF2,*

DAPL1, RPL34, CWC15, NDUFB3, RPL26L1, ACOT13, HSPB11, and NSA2. Some of them could have a potential role in vitiligo.

CXCL10 as a hub gene showed a higher expression level in lesional skin in comparison with non-lesional in vitiligo patients. *CXCL10* has been recognized as a chemokine that is induced by IFN-γ in several cell types, including lymphocytes, neutrophils, endothelial cells, and the other epithelial cells.[22] *CXCL10* binds to its particular receptor, *CXCR3*, and controls immune responses by recruiting and activating T cells, natural killer (NK) cells, and monocytes.[22] In leukocyte homing, *CXCL10* plays a significant role in inflamed tissues, exacerbates inflammation, and causes significant tissue damage.[23] Due to the high expression level of *CXCR3* on auto-reactive T cells in both human vitiligo patients and animal models, as well as the requirement of *CXCR3* for auto-reactive T cell accumulation in the skin and subsequent depigmentation, it is suggested that this chemokine is involved in vitiligo pathogenesis. Besides, it has been recognized that cytotoxic T lymphocytes (CTLs) are the main factor attacking melanocytes in vitiligo. Both in peripheral blood and skin lesions, the number of CTLs is significantly increased.[17] Therefore, using small molecule inhibitors of *CXCR3* which are currently under development by multiple sources,[24] may be effective therapies. In a mouse model of vitiligo, the clear dominance of *CXCL10* over *CXCL9* is also intriguing, providing an opportunity to target CXCL10 alone for new treatments.[25,26] Accordingly, the result of the drug-target network (Figure 5) suggests that some food and drug administration (FDA) approved drugs including Atropine, Stavudine, Atorvastatin, Oxaliplatin, Ritonavir, Zidovudine, Gonadotropin, Testosterone, and Methylprednisolone can affect vitiligo disease status through regulation of *CXCL10*. For example, Testosterone as a sex hormone has marked immune-modulatory properties and may play important roles in the etiology of various autoimmune diseases.[27] Researchers found that Testosterone induces the suppression of Sjögren's syndrome.[28] Muto et al showed that vitiligo patients were successfully treated by oral administration of a sex steroid-thyroid hormone. The re-pigmented skin showed increased numbers of melanocytes and melanin granules in the keratinocytes.[29] Based on the drug-target network (Figure 5) which shows an interaction between Testosterone and *CXCL10* gene, Testosterone

can be suggested as a potential drug for vitiligo.

The other hub gene which could have a potential role in vitiligo is *RPL26* (Ribosomal Protein L26). Researchers proposed that the silencing of ribosomal protein L26 and L29 markedly decreased the intracellular ROS generation.[30] In our study, we found that *RPL26* expression was highly increased in the lesional compared to non-lesional skin.

NDUFB3 (NADH:Ubiquinone Oxidoreductase Subunit B3) has proved many different roles in several diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and in oxidative phosphorylation process.[31] Some reports implicated that, oxidative stress process and some genetic variations in oxidant/antioxidant genes are strongly correlated to vitiligo.[32] In addition, toxic components enhancement including catecholamine, 7-BH4 with antioxidant enzymes reduction is involved in the pathology of the patient's skin.[33] The DNA damage through an abnormality in melanocyte homeostasis is due to several reactive oxygen species and hydrogen peroxide.[34] Oxidative stress and autoimmune reactions have been suggested as significant mechanisms for initiating and controlling vitiligo.[35,36] Reactive oxygen species (ROS) can cause productions of chemokine to stimulate the infiltration of T-cells into inflammatory areas,[26] which may be a key factor in the pathogenesis of vitiligo. ROS accumulation can also induce lipid peroxidation, DNA damage, increased production of pro-inflammatory and anti-melanogenic cytokines, and loss of enzyme functionality in melanogenesis.[37] miRNAs play a critical role in regulating the pathophysiology of autoimmune diseases such as vitiligo. miRNAs and their targets may play important roles in vitiligo's oxidative phosphorylation and mitochondrial energy metabolism and have therapeutic and biomarker potential.[38] In our study, we evaluated the miRNAs which can target hub genes. For example, the result of the miRNA-mRNA bipartite network (Figure 6) indicated hsa-mir-939-3p and hsa-mir-612 targeted *CXCL10*. The previous studies have pointed out the role of hsa-mir-612 in autoimmune diseases, such as rheumatoid arthritis (RA). They proved significantly changes in the hsa-mir-612 expression level of peripheral blood mononuclear cells in healthy people compared with RA patients.[38] Also, researchers indicated miR-939 as a regulator in the multiple diseases and also it's predicted to target some pro-inflammatory genes, such as *IL-6*, *TNF-α*, *NFκB2*, and

nitric oxide synthase 2.[39] These miRNAs may have a critical role in vitiligo disease.

In summary, *CXCL10, ARL9, AKR1B10, COX7B, RPL26, SPA17, NDUFAF2, RPF2, DAPL1, RPL34, CWC15, NDUFB3, RPL26L1, ACOT13, HSPB11,* and *NSA2*, as candidate genes in vitiligo, were from the Turquoise module and the expression levels of them involved in pathologic stage of patients. All of these hub genes as cell cycle regulators, regulate and participate in the development and progression of vitiligo. Among them, many studies reported that *CXCL10*, *RPL26*, and *NDUFB3* played main and important roles in the progression vitiligo, and others were showed an unclear mechanism of vitiligo pathogenesis. Thus, the remaining 13 candidate genes which we examined can be considered as novel biomarkers or vitiligo therapeutic purposes. The outcomes of this study will provide new insight into the pathogenesis of vitiligo. Generally, based on the evidence and theories mentioned above, the immune system and oxidative stress have a complicated pathogenesis role in vitiligo. Indeed, evidence demonstrates that autoimmunity and oxidative stress work together to create a pathway that can finally determine melanocyte loss.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

REFERENCES

1. Spritz RA. The genetics of generalized vitiligo and associated autoimmune diseases. Pigment Cell Res. 2007;20(4):271-8.

2. Jadali Z, Eslami MB, Sanati MH, Mansoori P, Mahmoudi M, Maghsoodi N, et al. Identification of hitherto undefined B-cell epitopes by antibodies in the sera of vitiligo patients using phage-display peptide library. Iran J Allergy Asthma Immunol. 2003;2(4):197-201.

3. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, et al. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res.

2012;25(3):E1-13.

4. Silverberg NB. The Epidemiology of Vitiligo. Curr Dermatol Rep. 2015;4(1):36-43.

5. Richmond JM, Frisoli ML, Harris JE. Innate immune mechanisms in vitiligo: danger from within. Curr Opin Immunol. 2013;25(6):676-82.

6. Zhao Y, Srivastava D. A developmental view of microRNA function. Trends Biochem Sci. 2007;32(4):189-97.

7. Rezaei Z, Sebzari A, Kordi-Tamandani DM, Dastjerdi KJD, biology c. Involvement of the Dysregulation of miR-23b-3p, miR-195-5p, miR-656-5p, and miR-340-5p in Trastuzumab Resistance of HER2-Positive Breast Cancer Cells and System Biology Approach to Predict Their Targets Involved in Resistance. 2019;38(2):184-92.

8. Rebane A, Akdis CA. MicroRNAs: Essential players in the regulation of inflammation. J Allergy Clin Immunol. 2013;132(1):15-26.

9. Sonkoly E, Wei T, Janson PC, Saaf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One. 2007;2(7):e610.

10. Rebane A, Runnel T, Aab A, Maslovskaja J, Rückert B, Zimmermann M, et al. MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. J Allergy Clin Immunol. 2014;134(4):836-47.

11. Vennegaard MT, Bonefeld CM, Hagedorn PH, Bangsgaard N, Lovendorf MB, Odum N, et al. Allergic contact dermatitis induces upregulation of identical microRNAs in humans and mice. Contact Derm. 2012;67(5):298-305.

12. Shi YL, Weiland M, Lim HW, Mi QS, Zhou L. Serum mi RNA expression profiles change in autoimmune vitiligo in mice. Exp Dermatol. 2014;23(2):140-2.

13. Wang Y, Wang K, Liang J, Yang H, Dang N, Yang X, et al. Differential expression analysis of mi RNA in peripheral blood mononuclear cells of patients with non-segmental vitiligo. Int J Dermatol. 2015;42(2):193-7.

14. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE 75819.

15. Cao W, Wu W, Yan M, Tian F, Ma C, Zhang Q, et al. Multiple region whole-exome sequencing reveals dramatically evolving intratumor genomic heterogeneity in esophageal squamous cell carcinoma. Oncogenesis. 2015;4(11):e175.

16. Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. Pigment Cell Res. 2003;16(2):90-100.

17. van den Boorn JG, Konijnenberg D, Dellemijn TA, van der Veen JP, Bos JD, Melief CJ, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. The Journal of investigative dermatology. 2009;129(9):2220-32.

18. Ahmadloo S, Taghizadeh M, Akhiani M, Salimzadeh A, Keramatipour M. Single Nucleotide Polymorphism rs 2476601 of PTPN22 Gene and Susceptibility to Rheumatoid Arthritis in Iranian Population. Iran J Allergy Asthma Immunol. 2015;14(4):437-42.

19. Santamaria P. Cytokines and chemokines in autoimmune disease: an overview. Adv Exp Med Biol. 2003;520:1-7.

20. Arimilli S, Ferlin W, Solvason N, Deshpande S, Howard M, Mocci S. Chemokines in autoimmune diseases. Immunological reviews. 2000;177:43-51.

21. Khan U, Ghazanfar H. T Lymphocytes and Autoimmunity. International review of cell and molecular biology. 2018;341:125-68.

22. Antonelli A, Ferrari SM, Fallahi P. The role of the Th1 chemokine CXCL10 in vitiligo. Annals of translational medicine. 2015;3(Suppl 1):S16.

23. Liu M, Guo S, Stiles JK. The emerging role of CXCL10 in cancer (Review). Oncol Lett. 2011;2(4):583-9.

24. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med. 2014;6(223):223ra23.

25. Ogg GS, Rod Dunbar P, Romero P, Chen JL, Cerundolo V. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. J Exp Med. 1998;188(6):1203-8.

26. Yang L, Yang S, Lei J, Hu W, Chen R, Lin F, et al. Role of chemokines and the corresponding receptors in vitiligo: A pilot study. J Dermatol. 2018;45(1):31-8.

27. Van Vollenhoven RF, McGuire JL. Estrogen, progesterone, and testosterone: can they be used to treat autoimmune diseases? Cleve Clin J Med. 1994;61(4):276-84.

28. Vendramini AC, Soo C, Sullivan DA. Testosterone-induced suppression of autoimmune disease in lacrimal tissue of a mouse model (NZB/NZW F1) of Sjogren's syndrome. Invest Ophthalmol Vis Sci. 1991;32(11):3002-6.

29. Muto M, Furumoto H, Ohmura A, Asagami C. Successful treatment of vitiligo with a sex steroid-thyroid hormone mixture. J Dermatol. 1995;22(10):770-2.

30. Li C, Ge M, Yin Y, Luo M, Chen D. Silencing expression of ribosomal protein L26 and L29 by RNA interfering

inhibits proliferation of human pancreatic cancer PANC-1 cells. Mol Cell Biochem. 2012;370(1-2):127-39.

31. Kwon DN, Park WJ, Choi YJ, Gurunathan S, Kim JH. Oxidative stress and ROS metabolism via down-regulation of sirtuin 3 expression in Cmah-null mice affect hearing loss. Aging. 2015;7(8):579-94.

32. Laddha NC, Chatterjee S, Gani AR, Malek RA, Shah BJ, Begum R. Association of catalase T/C exon 9 and glutathione peroxidase codon 200 polymorphisms in relation to their activities and oxidative stress with vitiligo susceptibility in Gujarat population. Pigment Cell Res. 2007;20(5):405-7.

33. Schallreuter KU, Rubsam K, Gibbons NC, Maitland DJ, Chavan B, Zothner C, et al. Methionine sulfoxide reductases A and B are deactivated by hydrogen peroxide (H2O2) in the epidermis of patients with vitiligo. J Invest Dermatol 2008;128(4):808-15.

34. Moretti S, Fabbri P, Baroni G, Berti S, Bani D, Berti E, et al. Keratinocyte dysfunction in vitiligo epidermis: cytokine microenvironment and correlation to keratinocyte apoptosis. Histol Histopathol. 2009;24(7):849-57.

35. Bishnoi A, Parsad D. Clinical and Molecular Aspects of Vitiligo Treatments. Int J Mol Sci. 2018;19(5).

36. Rashighi M, Harris JE. Vitiligo Pathogenesis and Emerging Treatments. Dermatol Clin 2017;35(2):257-65.

37. Colucci R, Dragoni F, Moretti S. Oxidative stress and immune system in vitiligo and thyroid diseases. Oxid Med Cell Longev. 2015;2015:631927.

38. Shang Z, Li H. Altered expression of four miRNA (miR-1238-3p, miR-202-3p, miR-630 and miR-766-3p) and their potential targets in peripheral blood from vitiligo patients. J Dermatol. 2017;44(10):1138-44.

39. McDonald MK, Ramanathan S, Touati A, Zhou Y, Thanawala RU, Alexander GM, et al. Regulation of proinflammatory genes by the circulating microRNA hsa-miR-939. Sci Rep. 2016;6:30976.