Original Article

Identification of Candidate miRNAs and Predication of Their Role in Keratoconus

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Abstract

Background: Keratoconus (KTCN, OMIM 148300) is known as an eye degenerative disease leading to stromal thinning and conical shape of the cornea. These structural changes can be accompanied by loss of visual function in advanced cases. To date, in spite of recent advances in the investigation of molecular mechanisms which result in Keratoconus, there's still a lack of information about the role of miRNAs in this disorder. Accordingly, this study aims to find miRNA's aberrantly expression in KTCN suffering cases and to predict their role by investigating their possible interactions with significantly KTCN correlated genes.

Material and Methods: The RNA sequencing dataset was retrieved from GEO databases (http://www.ncbi.nlm.nih.gov/geo). The data were comprised of 25 normal and 25 KTCN cases. Weighted gene co-expression network analysis approach was used to construct a protein-coding gene co-expression network and investigate the significant modules. Gene with the higher module membership (MM) and gene significance (GS) in the selected modules were supposed to be more KTCN relevant genes. Then CluGO plugin in Cytoscape software was used for enrichment analysis of genes in the selected modules. Differentially expressed genes in KTCN cases compared with normal cases were obtained using the edgeR package in R. All experimentally recorded miRNA-mRNA interactions were downloaded from miRTarbase database. Possible interactions from differentially expressed miRNAs and genes included in significant modules were retrieved.

Results: Totally 2492 protein-coding genes (PCGs) and 99 miRNAs were up-regulated and 213 PCGs and 31 miRNAs were down-regulated. Significant correlation with the KTCN was observed in three modules, including brown, green-yellow, and salmon from the total of 15 modules. Genes in significant modules have been enriched to gene expression regulation related biological processes such as negative regulation of protein secretion, intra-Golgi vesicle-mediated transport, regulation of mRNA 3'-end processing, and cytoskeleton related gene ontologies such as modulation of the mitochondrial cytoskeleton. Up-regulated miRNAs that interact with down-regulated mRNAs within significant modules include miR-1305, miR-544a, miR-1245a, miR-4635, miR-4266.

Conclusion: Just as the results revealed most of the deregulated genes were involved in gene expression regulation processes. Therefore the de-regulated miRNAs might involve in the aberrant expression of their targets. In this case, mentioning miRNAs in the results section can be considered as potential diagnostic or therapeutic biomarkers for KTCN.

Keywords: MiRTarbase; Keratoconus; Biomarke.

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Introduction

Keratoconus is considered a degenerative eye disorder accompanied by stromal thinning and conical shape of the cornea as a consequence. Structural changes in the corneal layers will result in optical alterations and ultimately leads to aberrations in visual function [1]. In advanced KTCN cases, due to progression of stromal thinning or scarring, surgery is essential. The general prevalence of this disease is estimated to be 1 in 2000 [1]. Both genetic and environmental factors such as; constant eye rubbing or contact lens wear might involve in KTCN initiation and progression. Since the KTCN is a complex and heterogeneous disease, the exact causes of that is not clear yet. Although a variety of genetic variations has been introduced to be in relation to KTCN, most of them are specific to particular families or populations [2]. Thus they can't be considered as general causes of this disease.

As mentioned earlier KTCN is considered a heterogeneous defect and alteration in a variety of genes in combination with environmental factors might cause the disease. In this case, most of the previous studies that have focused only on the small fractions of candidate genes are less likely to find the precise solution for KTCN problem [3]. On the other hand, some of the previous studies have chosen the Top-Down modeling approach and OMICS experimental techniques to get a deeper understanding of the molecular mechanism behind the disease [4]. For instance, Nielsen et al. observed 56 differentially expressed protein-coding (DEGs) genes containing 47 up-regulated and 9 down-regulated using the microarray technique evaluating 471 protein-coding genes. Among the DEGs they have reported, deregulation of lysyl oxidase (LOX) and TIMP3 have been observed in a variety of different studies [5]. A significant reduction in the expression level of TIPM1 which is an inhibitor of metalloproteinase was also reported in some previous literature.

From a biological perspective when we observe deregulation of expression we might have molecular aberration at any level of genes, Transcripts, or proteins [6]. Non-coding RNAs play a crucial role in gene expression regulation mostly at the post-transcriptional level [7]. Among Non-coding RNAs, miRNAs are small RNA molecules with an average length of 22 nucleotides. They can interact with 3′ UTR of target mRNA leading to mRNA degradation or translation repression. Only one miRNA can regulate the expression of hundreds of genes via interaction with their mRNA transcripts [8]. In this case, alteration in the expression of hundreds of genes might only be a consequence of miRNA deregulation. Moreover, some other features of these molecules such as being tissue-specific make them great potential biomarkers [9]. Despite the importance of studying miRNAs in the disease being caused by deregulation of genes expression; there's a lack of information about miRNAs role in Keratoconus. On the other hand just as mentioned above the Keratoconus is a multifactorial disease thus investigation of only several miRNAs and their targets would not be a great informative in comparison with a holistic RNA sequencing study. In order to assess the miRNAs' role in the specific biological process, it's logical to focus on their possible targets. In this case, miRNAs can have a regulatory influence on the processes that their targets are involved in. Moreover, co-expression network analysis is a popular

and informative method for investigating the molecular mechanism behind the biological phenomena. In this method, the genes with highly similar expression patterns will be clustered in a separate module. And the idea behind this kind of clustering is that highly co-expressed genes are very likely to be involved in the same biological process [10]. Thus by enrichment analysis of each module, the likely activated or inactivated process will be obtained.

Therefore this study is about to look at the KTCN at the OMICS level. By considering alterations in all transcripts and their possible co-expression we're about to get a better insight into the KTCN molecular mechanism. Significant modules involving in KTCN and also hub genes which can be considered as potential biomarkers will be introduced. In addition by evaluation of miRNAs expression level in KTCN cases in comparison with normal cases, differentially expressed miRNAs will be retrieved and their effect on the significant protein-coding module's gene will be predicted using miRTarbase database. On the other hand, there are various tools available for enrichment analysis of protein-coding genes. But there's still a lack of information about processes that non-coding RNAs are involved. Hence enrichment analysis of altered protein-coding gene processes plays role in KTCN can be estimated, on the other hand by considering the co-expression between the coding and non-coding genes mechanism that non-coding genes might take part can be predictable.

Material and Methods

Data retrieving

Gene Expression Profile

The expression data were retrieved from GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) under the accession number of GSE77938 [11], using "GEOquery" [12] package of R [13]. The data were raw count matrix which had been obtained by analysis of raw RNA-seq data. The Illumina HiSeq 1500 (https://www.illumina.com/) had been used for RNA-sequencing and the platform accession number is GPL18460.

Study Population and Demographical Information

Totally 50 cases participated in the mentioned project (GSE77938) comprised of 25 KTCN suffering cases and 25 normal cases. All study participants had been recruited and examined in the Department of Ophthalmology, Medical University of Warsaw, Poland. The 25 KTCN corneal tissues have been obtained from non-related Polish patients during a keratoplasty procedure for KTCN. The 25 non-KTCN corneas, used as controls, were collected from patients who were referred for corneal transplantation for different reasons, such as bullous keratopathy, corneal scarring, ulcers, and perforations. Categorization of the samples based on their gene expression profiles, using hierarchical clustering method accompanied with cases' clinical features heat-map is shown in Figure-1.

Data Pre-Processing and Diffrentiall Expression Analysis

The genes with a mean of expression value less than one were removed. Then read count matrix file was subjected to calculation the Trimmed Mean of M-values (TMM) [14] The distribution of the data was visualized using a box plot. To identify the gene type (e.g protein-coding or miRNA, etc.) of each

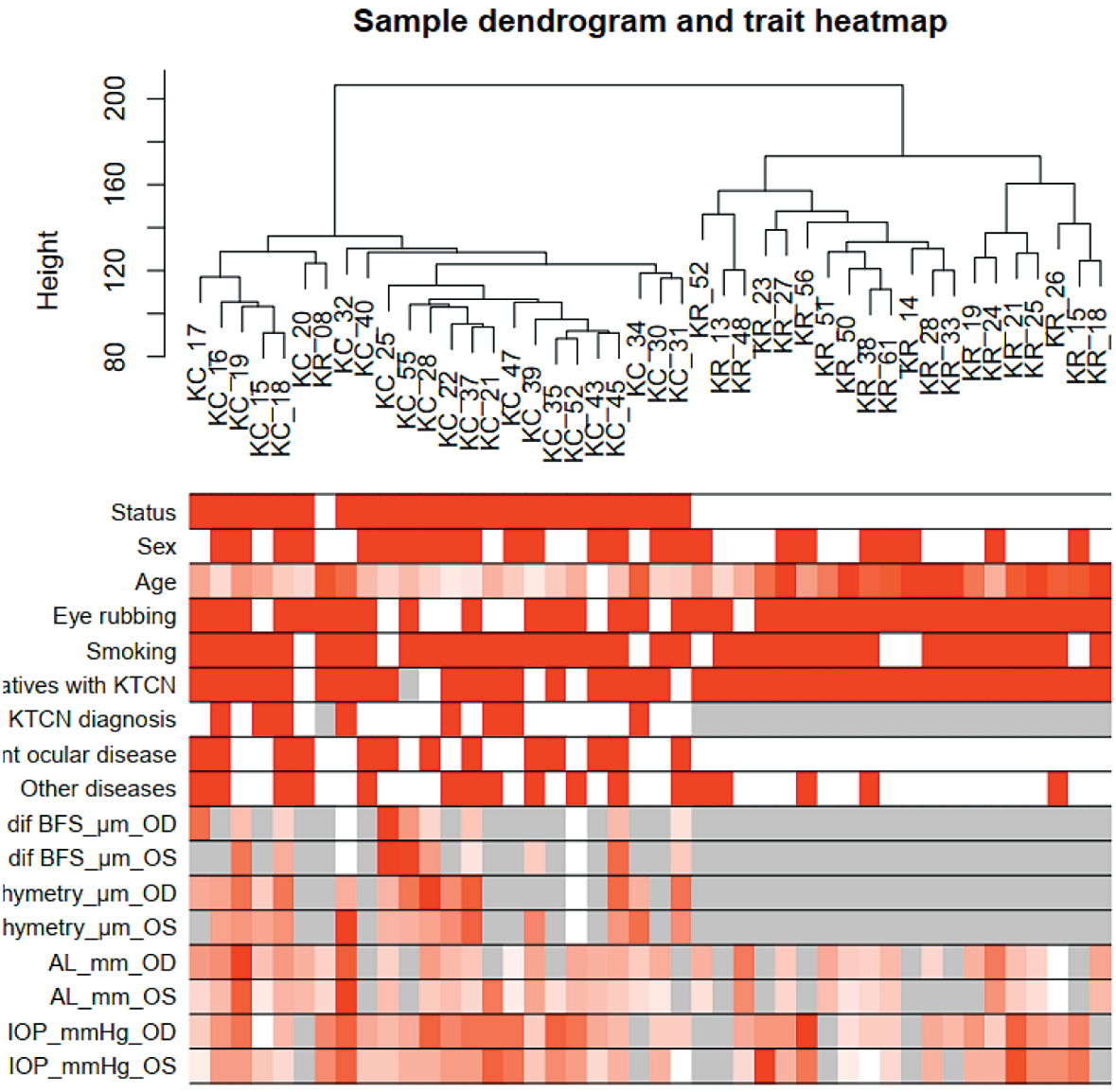

Figure 1: Hierarchical clustering of the samples based on the gene expression profile is illustrated above the figure. At the bottom, the heat map of clinical information of cases is illustrated

Ensembl ID, the annotation of human genome version 22 were downloaded from European Bioinformatics Institute (EMBL-EBI) (https://www.ebi.ac.uk/). Utilizing rtracklyr package in R gene type of each Ensembl ID were identified. After identification of gene types, the coding genes and miRNAs were separately filtered into two different matrices. Differentially expressed genes (DEGs) were identified using general linear modeling and p-values were adjusted using the false discovery rate method for each protein-coding gene matrix and miRNA matrix separately. All the process in DEGs identification and TMM normalization was performed using the edgeR [14] package of R [13]. In this package to moderate the degree of overdispersion across transcripts, empirical Bayes methods are used.

Selecting the gene with the highest variation in expression level among the samples

To focus on only the most important gens, we

took two steps.

1. The first step mentioned in the previous section and the genes with mean of expression value less than one were removed.
2. Only gene gens with the highest coefficient of variation (CV) were retained for further analysis.

CV can be calculated simply by dividing the Standard-Deviation of interest subjects by its mean; just as the Equation-1 shows:

$$CV = \frac{\sigma}{\mu} \quad 1$$

Since we're about to get a deeper view of the disease molecular mechanism, genes with the higher variation are more informative.

Network Construction and Module detection

Co-expression networks were constructed and analyzed, using the weighted gene co-expression network analysis (WGCNA) [15] package in R [13]. It has been approved that the WGCNA method is superior to other similar methods such as Partial Correlation and Information Theory [16]. The idea behind this method is that highly co-expressed genes can be clustered into the same network module which indicates their probable cooperation in the same biological process. Positively or negatively regulated genes can be clustered into different network modules since the type of the network selected to be signed [10]. To construct the network at the first step, a co-expression similarity matrix was calculated using Pearson correlation between each gene pair. Then the adjacency matrix will be computed by raising the similarity matrix to the soft threshold power of β. The β was chosen to be equal to 8 based on the scale-free criterion which has been proposed by [16] and resulted in a scale-free topology fit index (R^2) of 0.9 implying the network is biologically relevant. Moreover, the Topological Overlap Matrix (TOM) which is a similarity measure reflecting interconnectedness and neighbors overlap between nodes was calculated using the adjacency matrix. After that topological overlap dissimilarity matrix can simply be calculated by subtracting TOM from 1. This matrix is a good indicator of finding biologically meaningful modules. Hierarchical clustering of genes was performed based on the TOM dissimilarity matrix. Finally, the Dynamic Tree Cut algorithm [17] was used for identification of the network modules corresponding highly co-expressed genes.

To determine the most important modules the Module Eigengenes (MEs) were calculated for each module. The MEs are defined as the first principal component of its expression matrix. It's a summary of the expression profile for each module in only a one-dimensional vector. Then Module-Trait association was calculated using the MEs. To find possible relation between MEs and clinical traits both Spearman and Pearson correlations have been used. For comparison of MEs with categorical clinical traits such as having KTCN or not Sprearmant correlation and for MEs comparison with quantitative data such as age the Pearson correlation was used.

To candidate, the most important genes within each module two parameters have been considered. Module Membership (MM) is defined as the correlation of genes within each module with the module eigengene and Gene Significance (GS) which is a measure of the correlation of each gene module with the clinical traits. Gene with the highest GS and MM were introduced as the hub genes (MM & GS > 0.76 were selected).

miRNA-mRNA Interactions and Identification of Important miRNA

All experimentally validated mRNA-miRNA interactions were obtained from miRTARbase (https://mirtarbase.cuhk.edu.cn/) [18] database. Then only miRNA that was differentially expressed and mRNAs included in the module significantly associated with the KTCN were selected from the interaction. In this case, miRNA interacts with genes with the highest MM and GS are counted as the important miRNAs.

Gene Ontology and Pathway Enrichment Analysis

After the selection of modules significantly associated with KTCN, pathway enrichment and gene ontology (GO) analysis was performed on the gene involved in each module separately. The CluGO [19] application which is a Cytoscape [20] plugin was used to perform this analysis. KEGG (https://www.genome.jp/kegg/) database was used for pathway enrichment analysis. To calculate the significance of each biological term two-sided hypergeometric test was used and p-values were corrected by Benjamini & Hochberg method [21].

Results

Data Verification and Outlier Removal

Totally 60387 genes were included in the expression matrix. Genes with an average expression of less than 1 were excluded. 40842 genes remained after initial exclusion. Hierarchical clustering was performed on the samples based on their expression profile for outlier detection (Figure-1). From the total of 50 participants, two of the KTCN suffering cases (GSC, GSC) and 4 of the control cases were determined as outliers and removed.

Differentially Expressed Protein Coding Genes

After annotation of gene type and omitting the NA values total of 18518 protein-coding genes remained. The general linear model was used to identify differentially expressed genes than genes with the absolute Logarithm of fold change (|logFC|) ratios > 1.5 and adjusted p-value of < 0.01 were filtered and assigned as differentially expressed. The range of LogFC for protein-coding genes was between -5.6 and 13. Totally 2705 protein-coding genes were differentially expressed and among them 2492 were up-regulated and 213 were down-regulated. Table-1 shows the top 5 up and down-regulated genes.

Differentially Expressed miRNAs

After annotation and deleting the NA values 861 miRNAs were identified which among them 130 miRNAs were differentially expressed by absolute LogFC of greater than 1 and adjusted p-value less than 0.05. 99 miRNA were up-regulated and 31 were downregulated. After mapping ensemble ID to gene symbol of miRNAs only 70 miRNA remained. The top 5 up and down-regulated miRNAs are mentioned in Table-1.

Co-Expression Network Construction and Module Detection

The genes with the highest CV were used as input of the WGCNA method to construct a co-expression network. Hierarchical clustering of 4126 selected genes and the resulting modules were performed by Dynamic Tree Cut algorithm and illustrated in Figure-3. Totally 14 modules containing highly co-express genes were demonstrated and each module was

Table 1: Top 5 up-regulated genes and top 5 down regulated genes were mentioned in the table. In addition, genes with highest module membership (MM) and gene signifcance (GS) for each signficant modules (Brown, Green-Yellow, Salmon) were included in the table. Fasle Discovery Rate (FDR)

	Ensembl ID	logFC	logCPM	LR	FDR	Gene_symbole
Up-regulated PCGs	ENSG00000254709	13.41846	3.656764	73.73703	1.55E-16	IGLL5
	ENSG00000237172	10.04976	0.310891	97.55116	1.73E-21	B3GNT9
	ENSG00000243509	9.360745	-0.37629	71.1165	5.47E-16	TNFRSF6B
	ENSG00000132465	9.01506	2.346683	91.2894	3.46E-20	JCHAIN
	ENSG00000143556	8.843518	2.724701	45.79551	1.15E-10	S100A7
Down-regulated PCGs	ENSG00000144820	-5.65956	1.449844	29.3037	3.38E-07	ADGRG7
	ENSG00000125788	-4.11659	-3.83468	38.73039	3.51E-09	DEFB126
	ENSG00000185736	-4.09826	7.754363	93.07002	1.47E-20	ADARB2
	ENSG00000188710	-3.38655	-1.00114	25.36155	2.25E-06	QRFP
	ENSG00000130720	-3.35525	3.250862	81.1872	4.32E-18	FIBCD1
Up-regulated miRNAs	ENSG00000207569	5.722399	8.952251	48.62843	1.33E-10	hsa-miR-433
	ENSG00000208027	5.326938	5.168176	57.98944	3.03E-12	hsa-miR-485
	ENSG00000207762	5.285145	5.16031	66.60494	1.43E-13	hsa-miR-329-2
	ENSG00000207993	5.261272	5.133412	62.0194	8.95E-13	hsa-miR-134
	ENSG00000207978	5.183554	5.09456	61.6229	8.95E-13	hsa-miR-154
Down-regulated miRNAs	ENSG00000212066	-2.80973	5.044221	30.75588	5.04E-07	hsa-miR-1185-1
	ENSG00000198997	-2.50274	4.76327	26.05217	4.85E-06	hsa-miR-5739
	ENSG00000221519	-1.81962	4.856879	16.11417	0.000604	hsa-miR-3065
	ENSG00000265423	-1.64656	5.242883	12.24422	0.003978	hsa-miR-7845
	ENSG00000221402	-1.61405	15.00861	26.69237	3.54E-06	hsa-miR-107
Brown Module	Ensembl ID	MM	MM_p-value	GS	GS_p-value	Gene_symbole
	ENSG00000111707	0.870637	1.60E-14	0.865354	3.52E-14	SUDS3
	ENSG00000131381	0.883596	1.99E-15	0.865354	3.52E-14	RBSN
	ENSG00000006744	0.874169	9.28E-15	0.861771	5.89E-14	ELAC2
	ENSG00000145191	0.865001	3.70E-14	0.861771	5.89E-14	EIF2B5
	ENSG00000139697	0.847342	4.09E-13	0.858187	9.72E-14	SBNO1
Green-Yellow Module	ENSG00000160049	0.853015	1.96E-13	0.840271	9.84E-13	DFFA
	ENSG00000115073	0.762057	1.87E-09	0.836688	1.51E-12	ACTR1B
	ENSG00000140350	0.840246	9.87E-13	0.833105	2.30E-12	ANP32A
	ENSG00000167186	0.781746	3.75E-10	0.811605	2.34E-11	COQ7
	ENSG00000103423	0.896485	1.93E-16	0.808022	3.35E-11	DNAJA3
Salmon Module	ENSG00000108587	0.788757	2.04E-10	0.840271	9.84E-13	GOSR1
	ENSG00000114491	0.901323	7.40E-17	0.815188	1.62E-11	UMPS
	ENSG00000162521	0.892157	4.36E-16	0.797272	9.39E-11	RBBP4
	ENSG00000023228	0.824563	6.00E-12	0.793689	1.31E-10	NDUFS1

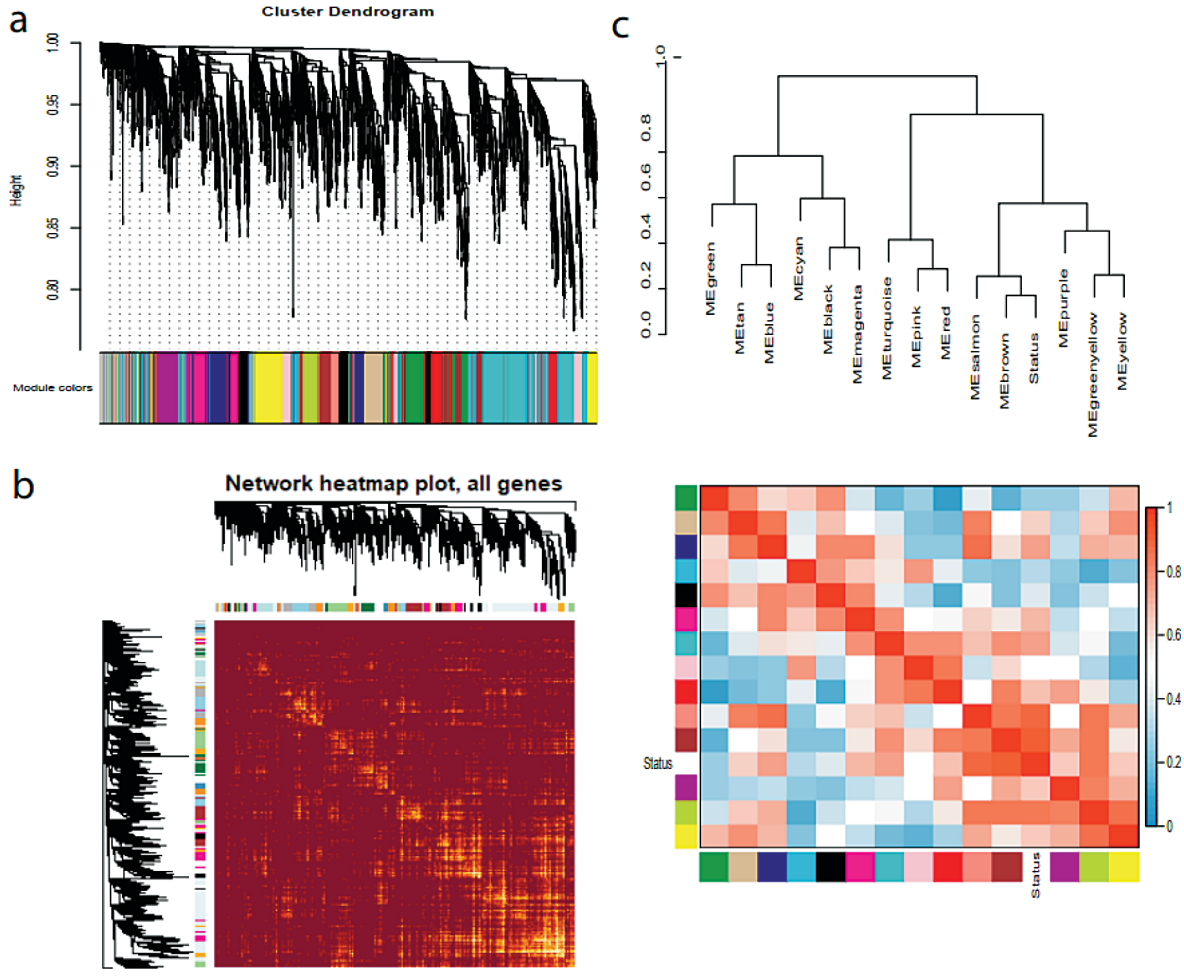

Figure 2: The cluster of genes and the modules that they're involved in is shown in (a). The correlation between all selected genes for network construction is illustrated in (b). The hierarchical clustering of module eigengenes can be seen in (c) above segment and the heatmap of module-module correlation can be seen in (c) bottom section

assigned a specific color. In Figure-3 the heat-map of gene-gene correlation and is shown. The heat map of inter-module correlation is also illustrated in Figure-3 accompanied by Hierarchical clustering of MEs.

In order to find significant modules, their (MEs') correlation with the clinical traits has been calculated. As shown in Figure-4 brown, salmon and green-yellow modules were significantly (p-value < 0.01) correlated with the status of having KTCN. There is no correlation between other clinical traits and any module; except a negative significant correlation between the brown module and the participants' age (p-value < 0.01). Genes with the highest MS and GS for each module have been mentioned in Table-1.

Interactions Between miRNAs & mRNAs

All experimentally validated miRNAs possessing the ability to target mRNAs involved in one of the three significant modules including brown, green-yellow, and salmon have been retrieved from miRTARbase

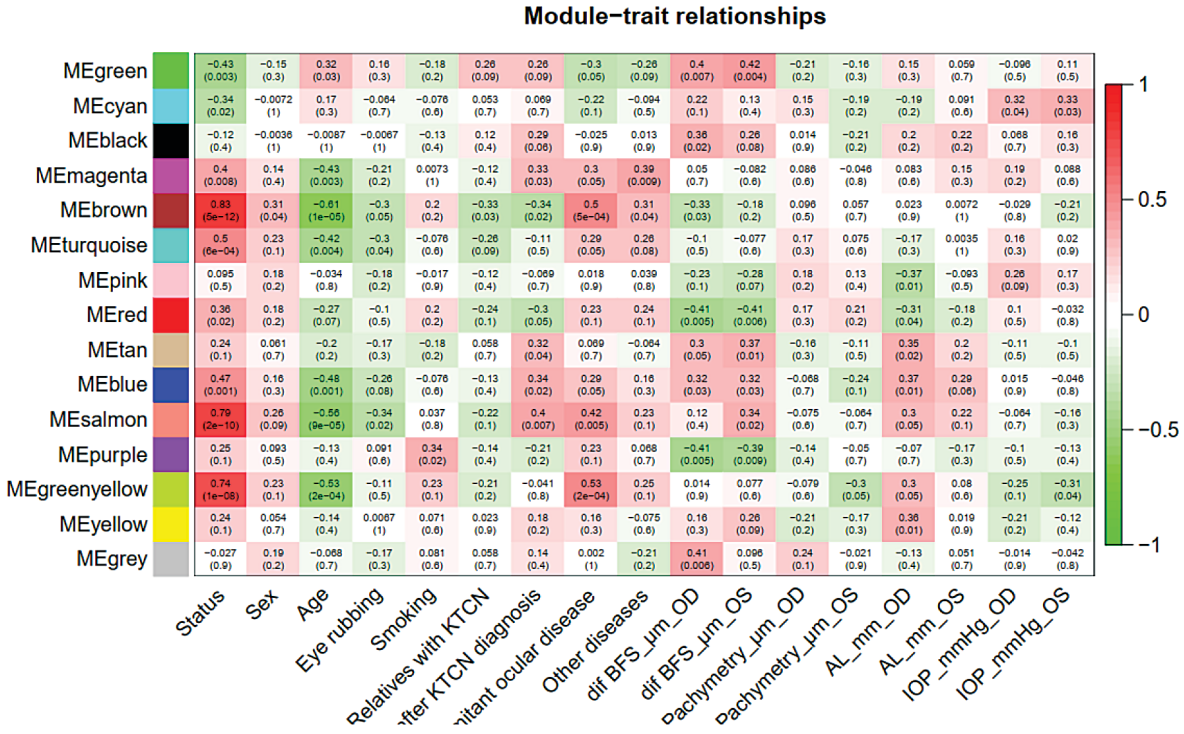

Figure 3: The correlation of module eigengenes and the clinical information of the cases is illustrated in a heatmap

database. Figure-4 shows miRNA-mRNA interactions within significant modules. The miRNAs have been shown with triangles and mRNAs have been shown with circle shapes. Up-regulated genes are shown with red and down-regulated genes with blue. The size of each node indicates its GS. As it can be seen in Figure-4 most of the time up-regulation of a specific miRNA is associated with the down-regulation of the target gene.

Gene Set Enrichment Analysis

Genes, included in significant modules were recruited for enrichment analysis. Gene ontology of the genes including their molecular function, the biological process was investigated. Moreover, KEGG database was used, for finding the pathways that the gene might be involved in. Brown module containing 329 protein-coding genes, participate in the biological process such as modulation of the mitochondrial cytoskeleton, positive regulation of the cellular catabolic process, methyltransferase activity, etc. The 184 genes that made up the green-yellow module take part in the biological process including, nucleoside bisphosphate biosynthetic process, spliceosomal snRNP assembly, nucleolar large RNA transcription by RNA polymerase I. Finally, the salmon module with the 78 genes comprising it is involved in, negative regulation of protein secretion, intra Golgi vesicle-mediated transport, regulation of mRNA 3'-end processing.

Discussion

As mentioned earlier the Keratoconus is a rather prevalent eye disorder that might lead to loss of fair visual function [1]. In spite of

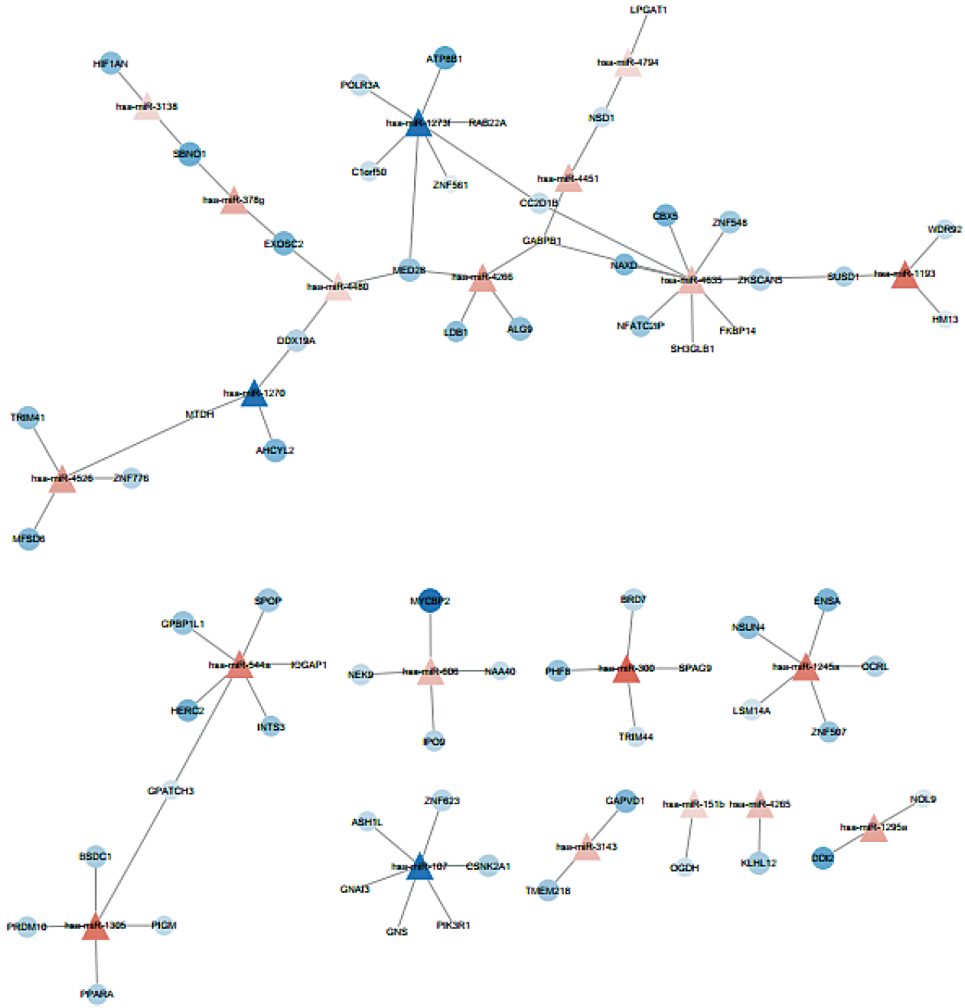

Figure 4: Interaction differentially expressed miRNAs and genes of the brown module is illustrated. The triangles and circles show miRNAs and mRNAs respectively. The size of each circle indicates its gene significance. The blue color shows down-regulation and the red color shows up-regulation and the intensity of the color is correlated with the rate of fold change in expression

extensive previous studies, the exact molecular mechanism causing this disease and especially the role of non-coding RNA remained to be understood [2,4]. Accordingly in this project via recruitment of publicly available RNA-seq data-set (GSE77938) [11] we tried to take a step further in a deeper understanding of the disease. At first, we looked for differentially expressed genes; the top 5 up & down-regulated genes can be found in Table-1. For instance, from the up-regulated genes the anti-microbial effect of S100A7 was proved in several previous studies, and also its up-regulation for reported in Keratoconus cases [22–25]. Aberrant expression of collagen formation related genes that have been reported in previous studies [11] to be associated with KTC, was also observed in our study, for instance, COL6A1 was significantly up-regulated (p-value < 0.001).

In the next step, the WGCNA approach was used to construct a co-expression network. Furthermore to candidate the significant

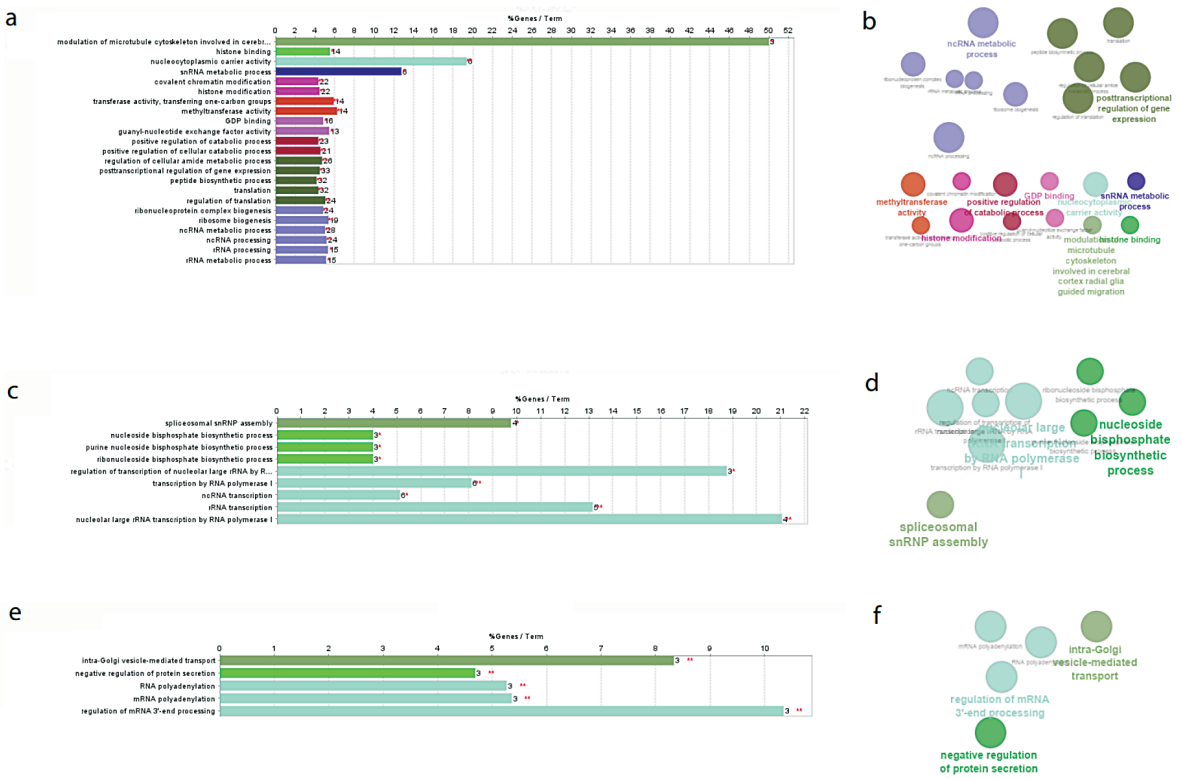

Figure 5: Biological process and pathways is illustrated in the figures in both bar plots (right) and networks (left)

modules and prioritize candidate genes the correlation of MEs with clinical traits and Gens were calculated respectively. Just as mentioned earlier three-module showed a significant correlation with the KTCN. Gene with the highest MM and GS for each module separately can be found in Tabl-1. For instance, SUDS3 in module brown was reported to be associated with ultrasound-derived measurements of the rib-eye area in Nellore cattle [26]. As Figure-5 shows the brown module's gene is mostly involved in the non-coding RNA metabolic process, post-transcriptional regulation of gene expression, modulation microtubule cytoskeleton involved in cerebral cortex radial glia guided migration, etc. If we look at KTCN from an upper angle it's a disease caused by deregulation of genes, especially Collagen related pathways. In this case, most of the enriched process of the brown module is related to the regulation of gene expression such as non-coding RNA metabolic process, post-transcriptional regulation of gene expression. Furthermore, some of them are involved in the modulation of microtubule cytoskeleton that can be directly relevant to KTC. The Salmon module's gene also was strongly related to gene expression regulation, especially at the protein level. For instant intra-Golgi vesicle-mediated transport, negative regulation of protein secretion, and so on. In confirmation of our hypothesis the green-yellow modules' genes show to be involved in processes such as spliceosomal snRNP

assembly nucleolar large RNA transcription by RNA polymerase I, which all are part of gene expression regulation; more details are illustrated in Figure-5.

In addition, we assessed differentially expressed miRNAs in KTCN cases in comparison to controls. Since it's proved that up-regulation of a miRNA can be directly associated with the down-regulation of several mRNAs, we tried to identify the possible interaction of differentially expressed miRNA and mRNA included in the significant modules. Accordingly up-regulated miRNAs that are capable to interact with down-regulated mRNAs in one of the significant modules are pretty much likely to be involved in the de-regulation of KTCN. Figure-4 illustrates mRNA-miRNA interactions.

For instance, each of these miRNAs; miR-1305, miR-544a, miR-1245a, miR-4635, miR-4266; can interact with several down-regulated genes with high GS in brown modules. Hence they can be considered as potential important miRNAs associated with KTCN by playing a regulatory role in biological processes such as; modulation of the microtubule cytoskeleton, non-coding RNA metabolic process, post-transcriptional regulation of gene expression.

Conclusion

The results of this study could provide us information about candidate miRNA and their possible role in Keratoconus for the first time. For instance according to the results miR-1305 can down-regulate several genes participating in keratoconus including BSDC1, PIGM, PPARA, PRDM10 (Figure-4). Just as Figure 4 shows the up-regulated miRNAs in keratokonus cases including miR-1305, miR-544a, miR-1245a, miR-4635, miR-4266 are palying role in down-regulation of genes involving in keratokonus. The mentioned miRNA might be potential diagnostic or therapeutic biomarkers. Thus the further experimental evaluation of these miRNAs in Keratoconus is suggested.

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References

1. Rabinowitz YS. Keratoconus. Vol. 42, Survey of Ophthalmology. 1998. p. 297–319.
2. Abu-Amero KK, Al-Muammar AM, Kondkar AA. Genetics of Keratoconus: Where Do We Stand? Journal of Ophthalmology. 2014.
3. Galvis V, Sherwin T, Tello A, Merayo J, Barrera R, Acera A. Keratoconus: An inflammatory disorder? Vol. 29, Eye (Basingstoke). 2015. p. 843–59.
4. Macé M, Galiacy SD, Erraud A, Mejía JE, Etchevers H, Allouche M, et al. Comparative transcriptome and network biology analyses demonstrate antiproliferative and hyperapoptotic phenotypes in human keratoconus corneas. Investig Ophthalmol Vis Sci. 2011;52(9):6181–91.

5. Nielsen K, Birkenkamp-Demtröder K, Ehlers N, Orntoft TF. Identification of differentially expressed genes in keratoconus epithelium analyzed on microarrays. Investig Ophthalmol Vis Sci. 2003;44(6):2466–76.
6. Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA Modifications in Gene Expression Regulation. Vol. 169, Cell. 2017. p. 1187–200.
7. Wei JW, Huang K, Yang C, Kang CS. Non-coding RNAs as regulators in epigenetics (Review). Oncol Rep. 2017;37(1):3–9.
8. Pu M, Chen J, Tao Z, Miao L, Qi X, Wang Y, et al. Regulatory network of miRNA on its target: coordination between transcriptional and post-transcriptional regulation of gene expression. Vol. 76, Cellular and Molecular Life Sciences. 2019. p. 441–51.
9. Silva SS, Lopes C, Teixeira AL, Sousa MJC De, Medeiros R. Forensic miRNA: Potential biomarker for body fluids? Vol. 14, Forensic Science International: Genetics. 2015. p. 1–10.
10. Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol. 2005;4(1).
11. Kabza M, Karolak JA, Rydzanicz M, Szcześniak MW, Nowak DM, Ginter-Matuszewska B, et al. Collagen synthesis disruption and downregulation of core elements of TGF-β, Hippo, and Wnt pathways in keratoconus corneas. Eur J Hum Genet. 2017;25(5):582–90.
12. Sean D, Meltzer PS. GEOquery: A bridge between the Gene Expression Omnibus (GEO) and BioConductor. Bioinformatics. 2007;23(14):1846–7.
13. R Development Core Team. R: A language and environment for statistical computing. Sci Rep. 2003;
14. Robinson MD, McCarthy DJ, Smyth GK. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2009;26(1):139–40.
15. Langfelder P, Horvath S. WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9.
16. Kadarmideen HN, Watson-haigh NS. Building gene co-expression networks using transcriptomics data for systems biology investigations: Comparison of methods using microarray data. Bioinformation. 2012;8(18):855–61.
17. Langfelder P, Zhang B, Horvath S. Defining clusters from a hierarchical cluster tree: The Dynamic Tree Cut package for R. Bioinformatics. 2008;24(5):719–20.
18. Huang H-Y, Lin Y-C-D, Cui S, Huang Y, Tang Y, Xu J, et al. miRTarBase update 2022: an informative resource for experimentally validated miRNA–target interactions. Nucleic Acids Res. 2021;
19. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009;25(8):1091–3.
20. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software Environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504.
21. Haynes W. Benjamini–Hochberg Method. In: Encyclopedia of Systems Biology. 2013. p. 78–78.
22. Garreis F, Gottschalt M, Schlorf T, Gläser R, Harder J, Worlitzsch D, et al. Expression and regulation of antimicrobial peptide psoriasin (S100A7) at the ocular surface and in the lacrimal apparatus. Investig Ophthalmol Vis Sci. 2011;
23. Garreis F, Gottschalt M, Paulsen FP. Antimicrobial peptides as a major part of the innate immune defense at the ocular surface.

Dev Ophthalmol. 2010;
24. Mohammed I, Mohanty D, Said DG, Barik MR, Reddy MM, Alsaadi A, et al. Antimicrobial peptides in human corneal tissue of patients with fungal keratitis. Br J Ophthalmol. 2021;
25. Nielsen K, Vorum H, Fagerholm P, Birkenkamp-Demtröder K, Honoré B, Ehlers N, et al. Proteome profiling of corneal epithelium and identification of marker proteins for keratoconus, a pilot study. Exp Eye Res. 2006;82(2):201–9.
26. Santana MHA, Ventura R V., Utsunomiya YT, Neves HHR, Alexandre PA, Oliveira Junior GA, et al. A genomewide association mapping study using ultrasound-scanned information identifies potential genomic regions and candidate genes affecting carcass traits in Nellore cattle. J Anim Breed Genet. 2015;132(6):420–7.

Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.