Original Article

Proliferative Diabetic Retinopathy from a Network Biology Perspective

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Abstract

Background: Proliferative Diabetic Retinopathy (PDR) is the advanced version of Diabetic Retinopathy in which, new, fragile blood vessels can start to develop in the retina and into the vitreous, the gel-like fluid that fills the back of the eye.

Material and Methods: Here we study PDR from a whole system viewpoint in which network science is utilized for the system representation. Our objective is to explore the role of differentially expressed genes in the development of PDR. For this purpose, we have designed a framework in which the genes with high differential expression are identified and their PPI networks are regenerated. Next, influential dominating nodes are specified in the resulting network. With the enrichment analyses, the output set is validated and its role in the PDR is studied.

Results: These results suggest that the output gene set has a significant association with the disease of study. Additionally, we identify miRNAs regulating the transcription of genes inside the explored module as biomarkers affecting the progress of PDR.

Keywords: Diabetic Retinopathy; Network; Systems Biology; Differential Gene Expression.

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Introduction

Diabetic Retinopathy (DR), also known as diabetic eye disease [1], is a serious complication of diabetes that threatens vision. It reasons progressive damage to the retina, the light-sensitive lining at the back of the eye. Initially, diabetic retinopathy might have no symptoms or only mild vision problems. However, over time it can go toward blindness. The condition can progress in everybody who has type 1 or type 2 diabetes. The longer diabetes and the less controlled blood sugar, the more probable this eye complication. DR affects up to 80 % of people who have suffered from diabetes for at least 20 years [2]. Diabetic retinopathy generally affects both eyes.

Non-proliferative diabetic retinopathy (NPDR) is the initial stage of the disease in which symptoms are slight or absent. In NPDR, the blood vessels in the retina are weakened. Tiny bulges in the blood vessels, called micro-aneurysms, can leak fluid inside the retina. On the other hand, Proliferative Diabetic Retinopathy is the more progressive. At this stage, circulation problems deny the retina of oxygen. Then, new, fragile blood vessels can start to develop in the retina and into the vitreous, the gel-like fluid that fills the back of the eye. Blood may be leaked into the vitreous by the new blood vessels, shadowing vision.

Over the years, many scientists have researched to identify the function and basis of the disease, to determine the causes of the disease and its progression, and to move towards treatment or prevention of disease exacerbation. These researches include either experimental [3-9] or computational [10-12] methods. However, as the role of engineering in biological phenomena becomes more prominent and more high-throughput generated datasets become available, the number of systemic approaches is increasing. In this context, their goal is to investigate the event from a holistic viewpoint. Improved comprehension of the biochemical structures underlying PDR can provide therapeutic approaches with the potential to be both impressive and less destructive than present methods.

Systems biology has brought us a variety of computational tools [13-15]. This approach successfully helps researchers to investigate molecular mechanisms of inhibition and activation of the genes and transcription factors, predict drug-target interactions, and detect novel molecular biomarkers of phenotypes [16-18]. Many RNA-RNA, RNA-miRNA, and RNA-Lnc-RNA interaction beside other macromolecules interactions in the cells; have been detected in the light of systems biology method [19-21].

Here we used the network biology [22] approach to map the biomolecules involved in PDR to a network model, then study its foundation and structure using computational methods. In this research, the RNAseq transcriptomic datasets were used to capture genes with variation in their expression levels in contrast to a normal state, and then analyze their relations in post-transcriptomic levels through computational auxiliaries.

Material and Methods

The used data here is adapted from [23] (accession number: GSE102485) and it contains a total of 30 transcriptome profiles of neovascular proliferative membrane specimens from 25 patients with PDR , 2 patients of type 2 diabetes, and 3 normal samples. Given that our goal is to study PDR disease, here we have used 25 PDR samples (as the case) and the 3 normal samples (as control). It is mentioned that all PDR patients of the study were in the fifth pathological stage. The high-throughput total

RNAseq data were preprocessed considering the criteria that genes must have Counts per Million (CPM) > 1 in more than two samples. The count values were normalized with the Trimmed Mean of M-values (TMM) normalization.

Differential Gene Expression analysis

To determine genes with differentiation in their expression level, we performed Differential Gene Expression (DGE) analysis among the 25 PDR disease samples and 3 healthy samples. Here, the R programming language with packages Limma [24] and edgeR [25] were utilized. We set the absolute logarithm of fold change to be larger than two (|LogFC| > 2) and an adjusted P value of less than 0.05 (adj. P value < 0.05).

Network Reconstruction

In order to model the disorder in terms of a biological graph, the differentially expressed genes (DEGs) were used to extract functional PPI subnetwork. The idea used here is that proteins translated from DEGs, destabilize the normal condition and cause abnormalities at post-transcriptional levels. For this reason, we extracted the experimentally curated PPI interactions of the examined DEGs (confidence level = 0.7, meaning of network edge = 'evidence', and active interaction source = 'databases' or 'experiments') from the STRING [26] database.

This subnetwork contains some Connected Components (CC), which are considered effective modules in disease development according to their tight interactions. From the CC set, the Largest Connected Component (LCC) is selected for further assessments.

Dominating Set Identification

Inside the LCC of the resulting network, we discovered the controlling proteins using the Dominating Set (DS) analysis. In an undirected graph with V nodes and E edges, a dominating set D is a subset of nodes () in a way that other nodes (are linked to at least one of the D set nodes. These nodes in some way are controlling the whole network as they have interactions with the entire network. We are concerned in genes that not only they are differentially expressed, but also their translated proteins are housed in the core of the PPI subnetwork, and they are the subnetwork controllers. Differentiation in their expression will change the behavior of the complete subsystem. To compute these driver nodes, we utilized the Networkx Python library which implemented it based on the algorithm [27].

Functional Enrichment

To confirm the functional activity of output DS, we employed the EnrichR [28] utility to discover the role of those genes in biological processes and pathways and their relation to the PDR disease. From the provided enrichment categories, for gene ontology, GO [29] Biological Processes, GO Molecular Function, GO Cellular Component, and for pathway enrichment, the KEGG [30], and for the disease overlap, data category DisGeNet [31] were used. Here also, we considered terms passing the condition of adj. P value < 0.05.

The pipeline applied in this research is presented in Figure 1.

Results

Data Statistics

Preliminary data used in this study included 30 samples (25 PDR patients + 3 healthy individuals + 2 type II diabetes specimens) containing 45952 transcripts. After the filtration process, 17,607 transcripts remained.

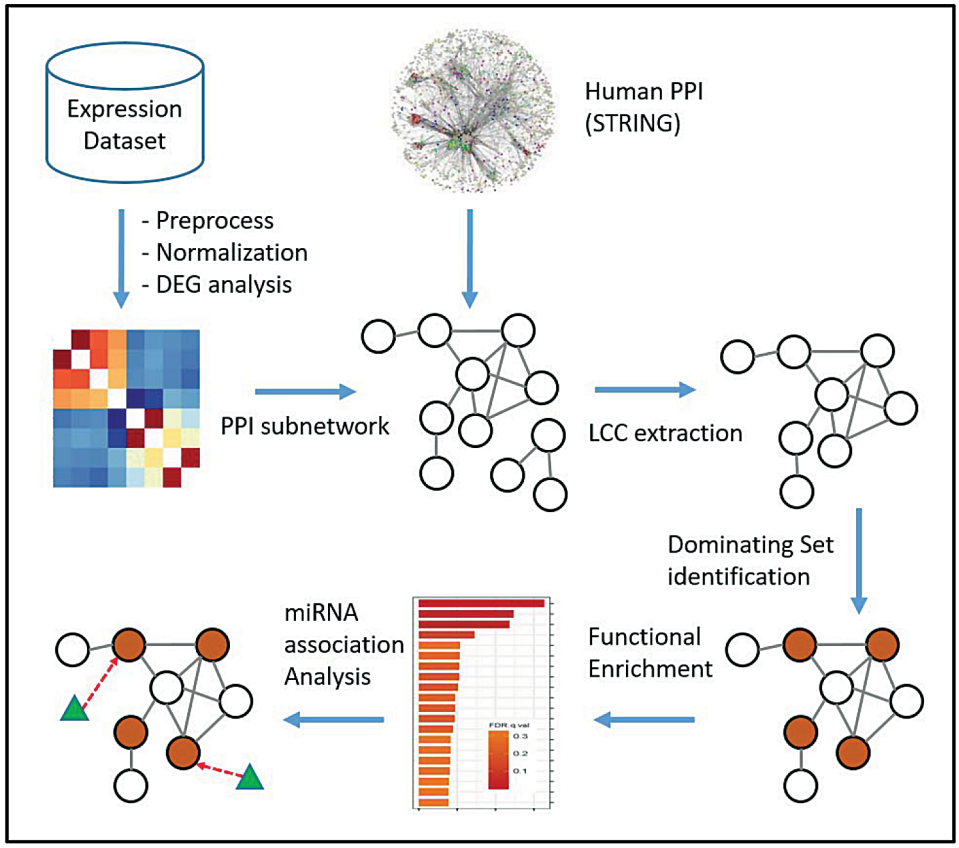

Figure 1: The followed pipeline in this research Materials and Methods

With the DGE analysis between the 25 PDR samples and the 3 healthy samples, it was specified that 1835 proteins coding PCG and 10 miRNA are expressed differentially. As mentioned before, the PPI network of PCG DEGs was taken from experimentally curated interactions of the STRING database and assembled a network with topological properties displayed in Table 1. The output network is scale-free, which is a noticeable pattern for real-world biological networks. Moreover, the network visualization is provided by Gephi [32] software that illustrated in Figure 2.

Table 1: Topological properties of the PPI subnetwork of DEGs

Property	Value
Number of Nodes	441
Number of Edges	1042
Network Diameter	13
Network Density	0.011
Average Degree	4.726
Number of connected components	53
Modularity	0.763

Because of the unreliability exerted from human's incomplete understandings from the PPI interactions, to be robust in the network analysis, the LCC is used in which interactions are more complete. The LCC, obtained from the PPI subnetwork, contains 267 nodes, and 802 edges. This is supposed to be a functional module at the proteomics level affecting the

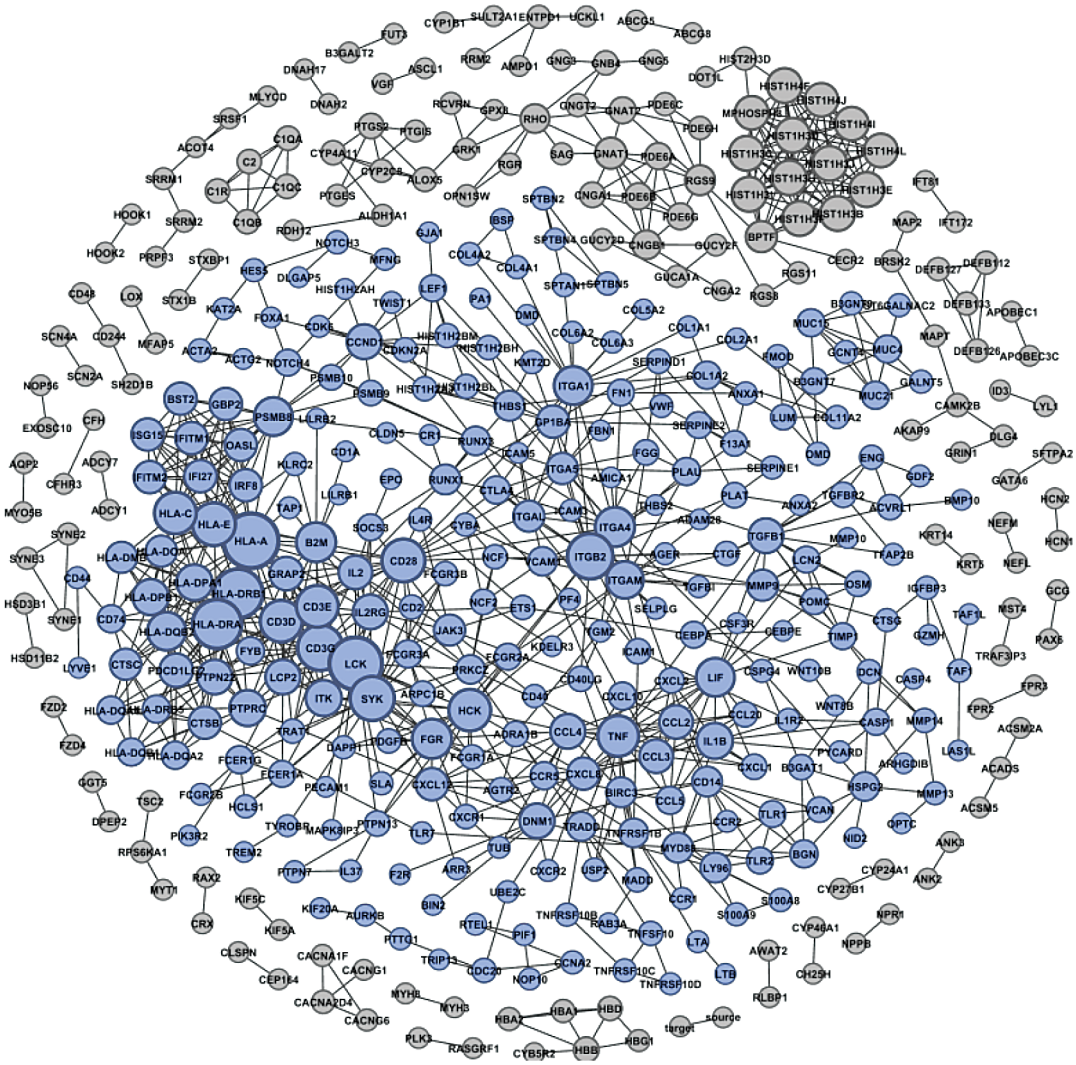

Figure 2: PPI subnetwork of DE genes. The blue module is the largest connected component

normal and stable condition. Variation in the expression of corresponding genes may play role in the progress of PDR. Inside the LCC module, we are looking for proteins with the most influence on others. This is addressed by the dominating nodes. Every node in the network (LCC) is linked to at least one dominating node. This means that any differentiation in their expression level will produce different quantities of their proteins, which have physical interactions with other proteins of the module. Therefore, these are good candidates to be studied. When the dominating set identification was applied, 109 nodes were specified as output set.

Functional Enrichment

We assessed the explored dominating nodes role in the related biological circles. Now, the intersection of discovered genes with diseases, pathways, biological processes, molecular functions, and cellular components is calculated statistically and the terms enriched in every test are provided in Tables 2, 3, 4, 5, and 6 respectively. In every table, the top enriched results are presented.

Table 2: Dominating set overlap with diseases

Term	P value	Adjusted P value	Odds Ratio	Combined Score
Inflammation	7.35E-18	1.74E-15	13.58501089	535.9545464
Chronic Lymphocytic Leukemia	5.72E-17	1.10E-14	7.849846532	293.5885133
Diabetic Retinopathy	9.27E-10	3.12E-08	11.3755787	236.5992408
Conventional (Clear Cell) Renal Cell Carcinoma	1.31E-09	4.28E-08	6.650344953	136.0431802
Nephritis	1.36E-09	4.36E-08	14.67360454	299.6243427
Infection caused by Helicobacter pylori	1.36E-09	4.36E-08	9.823057644	200.5781849
Malignant neoplasm of mouth	1.36E-09	4.36E-08	7.553135011	154.1767399
Diabetic Nephropathy	1.38E-09	4.37E-08	7.048023799	143.7976935
Lupus Erythematosus, Discoid	1.41E-09	4.44E-08	9.78925959	199.4780127
Proliferative vitreoretinopathy	1.59E-06	2.32E-05	19.25339806	257.080508
Osteopenia	1.85E-06	2.68E-05	6.908708767	91.21041873
Retinoblastoma	3.79E-05	4.09E-04	4.545628827	46.27682526
Proliferative diabetic retinopathy	5.59E-05	5.78E-04	13.61332418	133.3097151
Vitiligo	5.60E-05	5.78E-04	6.625152728	64.86721347
Proliferative retinopathy	8.75E-05	8.47E-04	19.39145299	181.2020794
Visually threatening diabetic retinopathy	8.07E-04	0.005037956	61.9470405	441.229228
Retinoschisis	0.001567	0.008502909	41.29179647	266.6723724

miRNA Association

Finally, we are interested in miRNAs regulating these functional gene set. For this reason, we inspected the differentially expressed miRNAs and their association with the dominating set. It is noticeable that the validated interactions of miRNA and their targets were adapted from miRTarbase (33) database. It was specified that from the 10 differentially expressed miRNAs, 4 (hsa-miR-21, hsa-miR-1304, hsa-let-7a-2, and hsa-miR-4477b) have inhibition link with 91 driver nodes. Therefore, any change in their expression will affect the whole module. As a result, we mark all 91 PCGs and four miRNAs as items to be evaluated experimentally.

Discussion

For the DR disease, it has been noticed that over time, too much sugar in the blood could lead to the blockage of the little blood vessels that feed the retina, cutting off its

Table 3: Dominating set overlap with Pathways

Term	P value	Adjusted P value	Odds Ratio	Combined Score
Hematopoietic cell lineage	6.36E-12	5.53E-10	25.26	651.22
Viral protein interaction with cytokine and cytokine receptor	1.75E-10	7.59E-09	22.22	499.33
Viral myocarditis	3.58E-08	7.78E-07	25.69	440.43
Allograft rejection	0.000001907	0.00002073	28.93	381.02
Cytokine-cytokine receptor interaction	4.50E-13	7.83E-11	13.04	370.62
Phagosome	6.87E-10	2.39E-08	15.72	331.71
Type I diabetes mellitus	0.000003578	0.00003662	25.12	314.99
Cell adhesion molecules	8.27E-09	2.06E-07	14.46	269.08
Intestinal immune network for IgA production	0.000006228	0.00005348	22.19	266
Malaria	0.00000764	0.00006043	21.2	249.82
ECM-receptor interaction	5.19E-07	0.000006452	16.78	242.89
Neutrophil extracellular trap formation	6.88E-09	2.00E-07	12.43	233.64
PI3K-Akt signaling pathway	8.88E-11	5.15E-09	9.95	230.34
AGE-RAGE signaling pathway in diabetic complications	0.00001795	0.0001201	12.27	134.07
B cell receptor signaling pathway	0.00008081	0.0004328	12.53	118.12
Mucin type O-glycan biosynthesis	0.0009863	0.003509	17.03	117.88
Human papillomavirus infection	2.58E-07	0.000004081	7.59	115.15
Leukocyte transendothelial migration	0.00003775	0.0002265	10.67	108.67
HIF-1 signaling pathway	0.02179	0.04799	5.28	20.21
Epithelial cell signaling in Helicobacter pylori infection	0.05593	0.1047	5.45	15.71

blood supply. Therefore, the eye develops new blood vessels. However, these fresh vessels do not grow appropriately and can leak simply. In line with this explanation, we examined the resulting dominant genes in more detail. First, the whole set is enriched in pathways: Type I diabetes mellitus (adj. P value = 0.00003662, genes: HLA-DRB5, LTA, HLA-C, IL2, and HLA-DQB1), AGE-RAGE signaling pathway in diabetic complications (adj. P value = 0.0001201, genes: COL1A1, CCND1, SERPINE1, PIK3R2, AGER, and ICAM1), HIF-1 signaling pathway (adj. P value = 0.04799, genes: EPO, SERPINE1 and PIK3R2), Mucin type O-glycan biosynthesis (adj. P value = 0.003509, genes: GALNT5, ST6GALNAC2, and GCNT4), and Cytokine-cytokine receptor interaction

Table 4: Go Biological processes of Dominating set

Name	P value	Adjusted P value	Odds Ratio	Combined score
regulation of dendritic cell differentiation (GO:2001198)	4.00E-07	0.00002258	94.68	1394.95
negative regulation of production of molecular mediator of immune response (GO:0002701)	0.00001291	0.0003398	93.8	1055.89
negative regulation of dendritic cell differentiation (GO:2001199)	0.0002912	0.003864	123.91	1008.85
atrial cardiac muscle tissue morphogenesis (GO:0055009)	0.0002912	0.003864	123.91	1008.85
negative regulation of immunoglobulin production (GO:0002638)	0.0002912	0.003864	123.91	1008.85
negative regulation of leukocyte differentiation (GO:1902106)	0.0002912	0.003864	123.91	1008.85
regulation of cell proliferation involved in heart morphogenesis (GO:2000136)	0.0002912	0.003864	123.91	1008.85
positive regulation of prostaglandin biosynthetic process (GO:0031394)	0.0002912	0.003864	123.91	1008.85
monocyte extravasation (GO:0035696)	0.0002912	0.003864	123.91	1008.85
negative regulation of hormone secretion (GO:0046888)	0.00001838	0.0004382	80.39	876.65

(adj. P value = 7.83E-11, genes: CCR1, BMP10, CSF3R, IL4R, EPO, LIF, OSM, TNFRSF10B, IL2, TNFRSF10D, CXCL12, CD40LG, CXCR1, CXCR2, LTA, CCR2, and PF4).

According to the KEGG, the majority of type 1 diabetes mellitus (T1DM) cases are believed to arise from an inflammatory, autoimmune attack against the beta cells in the pancreas, which subsequently leads to the failure of insulin-mediated blood glucose regulation in the body. This can lead to high blood sugar and the early stages of diabetic retinopathy.

Studies of the cellular and molecular components of hypoxia signaling have unlocked a novel era in the dealing of retinopathies. Any disruption of oxygen delivery can result in the development of some degenerative retinal diseases such as diabetic retinopathy. Agreed on the effect of HIF-1 in these disorders, it is obvious that any change of this pathway at different stages can result in effective treatment of oxygen-dependent eye diseases. The set of genes obtained in the present study shows that EPO, SERPINE1 and PIK3R2 genes (from HIF-1 signaling pathway) are stimulus nodes that play a key role in DR [25]. The set of genes obtained in the present study shows that EPO, SERPINE1 and PIK3R2 genes (from HIF-1 signaling pathway) are stimulus nodes that play a key role in DR [34].

O-glycans are a type of glycan that alters the residues of S (serine) or T (threonine) proteins. According to the joined sugars, there are four typical structures of O-glycan nuclei, nuclei 1 to 4, and the other four structures, nuclei 5 to 8. Mucins are extremely O-glycosylated

Table 5: Molecular functions of Dominating set

Name	P value	Adjusted P value	Odds Ratio	Combined score
MHC class Ib protein binding (GO:0023029)	0.000008644	0.0003112	112.56	1312.32
C-X-C chemokine binding (GO:0019958)	0.0002912	0.004193	123.91	1008.85
C-X-C chemokine receptor activity (GO:0016494)	0.0002912	0.004193	123.91	1008.85
platelet-derived growth factor binding (GO:0048407)	0.00002517	0.0005764	70.34	744.91
MHC class I receptor activity (GO:0032393)	0.0004352	0.00553	92.93	719.25
MHC class II protein binding (GO:0042289)	0.0004352	0.00553	92.93	719.25
MHC protein binding (GO:0042287)	3.90E-07	0.0000842	41.53	612.88
C-C chemokine receptor activity (GO:0016493)	0.000006824	0.0003112	39.84	473.94
C-C chemokine binding (GO:0019957)	0.000008154	0.0003112	37.85	443.48
superoxide-generating NADPH oxidase activator activity (GO:0016176)	0.0008066	0.008711	61.95	441.23

glycoproteins found in mucosal secretions at various levels from cellular to body fluids. Mucin O-glycans can be branched, and many sugars are antigens. Significant changes in mucin O-glycans contain o-acetylation of sialic acid, O-sulfate galactose, and N-acetylglucosamine.

AGE/RAGE signaling pathway in diabetic-mediated vascular calcification results in increased oxidative stress, leading to phenotypic changes in VSMCs to osteoblast-like cells in AGEs-induced calcification [26]. In addition, it enhances oxidative stress to promote diabetes-induced vascular calcification by activating Nox-1 and reducing SOD-1 expression.

Cytokine-cytokine receptor interaction is another pathway that was studied in our study. Cytokines are glycoproteins that play a fundamental role in the inter-cellular regulation of cells involved in differentiation, cell growth, angiogenesis, cell death, and growth and repair processes. Cytokines are usually diffused by different cells in response to a stimulus, and by binding to special receptors on the surface of the target cells, they induce a response. Cytokines can be classified into different series based on structure.

In a second step, we identified list of differentially expressed PCGs inside our dominating set targeted by differentially expressed miRNAs. The hsa-miR-21 regulates 44 PCGs in which TGFB1, CCND1, COL4A1, IL1B, ICAM1, and TGFBR2 are involved in the AGE-RAGE signaling pathway in diabetic complications (adj. P value = 0.000006094)

Table 6: Cellular Components of Dominating set

Name	P value	Adjusted P value	Odds Ratio	Combined score
collagen-containing extracellular matrix (GO:0062023)	1.37E-15	1.91E-13	12.98	444.39
lumenal side of endoplasmic reticulum membrane (GO:0098553)	1.5E-05	0.00027	31.53	349.34
integral component of lumenal side of endoplasmic reticulum membrane (GO:0071556)	1.5E-05	0.00027	31.53	349.34
MHC protein complex (GO:0042611)	0.00017	0.00196	33.09	287.62
MHC class II protein complex (GO:0042613)	0.00221	0.00997	33.78	206.6
apicolateral plasma membrane (GO:0016327)	0.02696	0.08986	46.03	166.35
phagolysosome (GO:0032010)	0.02696	0.08986	46.03	166.35
platelet alpha granule (GO:0031091)	9.8E-06	0.00023	13.74	158.42
bounding membrane of organelle (GO:0098588)	8.74E-10	4.08E-08	6.12	127.74
ER to Golgi transport vesicle membrane (GO:0012507)	0.00021	0.00215	15.12	127.73

that is proven pathway in the DR progress. Moreover, hsa-miR-21 targets CXCL10, CCL20, IL1B, and MMP9, taking part in the IL-17 signaling pathway. The IL-17A has emerged as an important inflammatory mediator involved in the genesis of immune and chronic inflammatory diseases, including cardiovascular and renal diseases and diabetic complications [35]. Additionally, it controls the TNF signaling pathway (adj. P value = 0.00009474). TNFα is required for Late BRB breakdown in diabetic retinopathy, and its inhibition prevents Leukostasis and protects vessels and neurons from apoptosis [36].

The amplified expression of hsa-miR-21 in PDR might be interrelated with encouraging angiogenesis through directing PTEN, resulting in the triggering of AKT and ERK1/2 signaling pathways, and thus improving VEGF and HIF-1α expression (Liu et al., 2011). Besides, the mentioned miRNA is highly expressed in response to high glucose and defends endothelial cells from apoptosis procedure.

In the same way, hsa-let-7a-2 regulates PDHA2, MAP7, KCNB1, GNAT1, and ACSM2A PCGs. PDHA2 related pathways are Glucose metabolism and Acetylcholine Synthesis. Gene Ontology remarks linked to this gene comprise acting on the aldehyde or oxo group of donors, disulfide as acceptor and pyruvate dehydrogenase, and oxidoreductase activity. Earlier studies confirmed that a profile of five serum miRNAs including hsa-let-7a-5p was meaningfully related with type 2 diabetes with diabetic retinopathy [37].

In our research, the other has-miR-1304 inhibits 42 protein-coding genes including PDHA2, HOXD8, TPBG, SOCS3, HLA-DRA, SERPINH1, PYCARD, FBXO47, SLC25A45, BTN3A2, EXOSC10, BDP1, SLC1A5, ZNF135, EMCN, PI4K2B, MAP7,

KIAA1614, TRIM38, IGSF6, MYO5B, LRRC3C, HIST1H2AH, PDE6A, RBM48, ACSL6, KRBA2, KIF3A, BPTF, GNB4, CABP4, GATA6, PSMB9, NNMT, APOL1, SLC2A5, UGT2B4, FGG, GPR132, CLDN1, SIGLEC9, and ZC2HC1C. Despite it is not reported as an involved microRNAs in the DR, its overexpression is stated in diabetes [38].

Finally, hsa-miR-4477b is the other candidate miRNA that regulates HIST1H3E, PTGDR, E2F8, and GNG5. Currently, there is not any specification for this miRNA and its association with the diabetic retinopathy.

Conclusion

In this research, we used a network-based approach to model proliferative Diabetic Retinopathy. Our goal was to study the disease event as a system and capture core drivers in the function level. Consequently, differentially expressed genes were specified and the network of their proteins' interactions was reconstructed. To be more confident in the current incomplete interactome, the largest connected component was selected to be studied. Dominating set of the LCC was assessed and functional enrichment results suggest that those genes are involved in pathways and processes related to PDR. Finally, we checked the miRNAs that target the dominating set and observed that four miRNAs, hsa-miR-21, hsa-miR-1304, hsa-let-7a-2, and hsa-miR-4477b, are candidates that affect their target expression.

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References

1. Li JQ, Welchowski T, Schmid M, Letow J, Wolpers C, Pascual-Camps I, et al. Prevalence, incidence and future projection of diabetic eye disease in Europe: a systematic review and meta-analysis. Eur J Epidemiol. 2020;35(1):11-23.

2. Kertes PJ, Johnson TM. Evidence-based eye care: Lippincott Williams & Wilkins; 2007.

3. Zhao Y, Singh RP. The role of anti-vascular endothelial growth factor (anti-VEGF) in the management of proliferative diabetic retinopathy. Drugs in context. 2018;7.

4. Sun JK, Glassman AR, Beaulieu WT, Stockdale CR, Bressler NM, Flaxel C, et al. Rationale and application of the protocol S anti–vascular endothelial growth factor algorithm for proliferative diabetic retinopathy. Ophthalmology. 2019;126(1):87-95.

5. Zhu X-R, Yang F-y, Lu J, Zhang H-r, Sun R, Zhou J-B, et al. Plasma metabolomic profiling of proliferative diabetic retinopathy. Nutr Metab (Lond). 2019;16(1):1-11.

6. Pan J, Chen D, Yang X, Zou R, Zhao K, Cheng D, et al. Characteristics of neovascularization in early stages of proliferative diabetic retinopathy by optical coherence tomography angiography. Am J Ophthalmol. 2018;192:146-56.

7. Shao Y, Dong L-j, Takahashi Y, Chen J, Liu X, Chen Q, et al. miRNA-451a regulates RPE function through promoting mitochondrial function in proliferative diabetic retinopathy. American Journal of Physiology-Endocrinology and Metabolism. 2019;316(3):E443-E52.

8. Wu F, Lamy R, Ma D, Laotaweerungsawat S, Chen Y, Zhao T, et al. Correlation of aqueous,

vitreous, and plasma cytokine levels in patients with proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci. 2020;61(2):26.
9. Suresh R, Hannah JY, Thoveson A, Swisher J, Apolinario M, Zhou B, et al. Loss to follow-up among patients with proliferative diabetic retinopathy in clinical practice. Am J Ophthalmol. 2020;215:66-71.
10. Qiao L, Zhu Y, Zhou H. Diabetic retinopathy detection using prognosis of microaneurysm and early diagnosis system for non-proliferative diabetic retinopathy based on deep learning algorithms. IEEE Access. 2020;8:104292-302.
11. Yang Y, Liu Y, Li Y, Chen Z, Xiong Y, Zhou T, et al. MicroRNA-15b targets VEGF and inhibits angiogenesis in proliferative diabetic retinopathy. The Journal of Clinical Endocrinology & Metabolism. 2020;105(11):3404-15.
12. Nagasawa T, Tabuchi H, Masumoto H, Enno H, Niki M, Ohara Z, et al. Accuracy of ultrawide-field fundus ophthalmoscopy-assisted deep learning for detecting treatment-naïve proliferative diabetic retinopathy. Int Ophthalmol. 2019;39(10):2153-9.
13. Mousavian Z, Díaz J, Masoudi-Nejad A. Information theory in systems biology. Part II: protein-protein interaction and signaling networks. Seminars in cell & developmental biology. 2016;51:14-23.
14. Mousavian Z, Kavousi K, Masoudi-Nejad A. Information theory in systems biology. Part I: Gene regulatory and metabolic networks. Seminars in Cell and Developmental Biology. 2016;51:3-13.
15. Torkamanian-Afshar M, Lanjanian H, Nematzadeh S, Tabarzad M, Najafi A, Kiani F, et al. RPINBASE: An online toolbox to extract features for predicting RNA-protein interactions. Genomics. 2020;112(3).
16. Masoudi-Sobhanzadeh Y, Omidi Y, Amanlou M, Masoudi-Nejad A. Trader as a new optimization algorithm predicts drug-target interactions efficiently. Scientific Reports 2019 9:1. 2019;9(1):1-14.
17. H L, S N, S H, M T-A, F K, M M-J, et al. High-throughput analysis of the interactions between viral proteins and host cell RNAs. Computers in biology and medicine. 2021;135:104611.
18. Aflakparast M, Salimi H, Gerami A, Dubé MP, Visweswaran S, Masoudi-Nejad A. Cuckoo search epistasis: a new method for exploring significant genetic interactions. Heredity 2014 112:6. 2014;112(6):666-74.
19. Ghasemi M, Seidkhani H, Tamimi F, Rahgozar M, Masoudi-Nejad A. Centrality Measures in Biological Networks. Current Bioinformatics. 2014;9(4):426-41.
20. Pournoor E, Elmi N, Masoudi-Nejad A. CatbNet: A Multi Network Analyzer for Comparing and Analyzing the Topology of Biological Networks. (1389-2029 (Print)).
21. Pournoor E, Mousavian Z, Dalini AN, Masoudi-Nejad A. Identification of Key Components in Colon Adenocarcinoma Using Transcriptome to Interactome Multilayer Framework. (2045-2322 (Electronic)).
22. Barabasi A-L, Oltvai ZN. Network biology: understanding the cell's functional organization. Nature reviews genetics. 2004;5(2):101-13.
23. Li Y, Chen D, Sun L, Wu Y, Zou Y, Liang C, et al. Induced expression of VEGFC, ANGPT, and EFNB2 and their receptors characterizes neovascularization in proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci. 2019;60(13):4084-96.
24. Smyth GK. Limma: linear models for microarray data. Bioinformatics and computational biology solutions using R and Bioconductor: Springer; 2005. p. 397-420.
25. Robinson MD, McCarthy DJ, Smyth GK.

edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139-40.
26. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2014;43(D1):D447-D52.
27. Esfahanian AH. Connectivity algorithms. Topics in structural graph theory: Cambridge University Press; 2013. p. 268-81.
28. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44(W1):W90-W7.
29. Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. Nucleic Acids Res. 2019;47(D1):D419-D26.
30. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27-30.
31. Taliun SAG, VandeHaar P, Boughton AP, Welch RP, Taliun D, Schmidt EM, et al. Exploring and visualizing large-scale genetic associations by using PheWeb. Nat Genet. 2020;52(6):550-2.
32. Bastian M, Heymann S, Jacomy M, editors. Gephi: an open source software for exploring and manipulating networks. Third international AAAI conference on weblogs and social media; 2009.
33. Huang H-Y, Lin Y-C-D, Li J, Huang K-Y, Shrestha S, Hong H-C, et al. miRTarBase 2020: updates to the experimentally validated microRNA–target interaction database. Nucleic Acids Res. 2020;48(D1):D148-D54.
34. Krishna Vadlapatla R, Dutt Vadlapudi A, Mitra AK. Hypoxia-inducible factor-1 (HIF-1): a potential target for intervention in ocular neovascular diseases. Curr Drug Targets. 2013;14(8):919-35.
35. Lavoz C, Rayego-Mateos S, Orejudo M, Opazo-Ríos L, Marchant V, Marquez-Exposito L, et al. Could IL-17A be a novel therapeutic target in diabetic nephropathy? Journal of clinical medicine. 2020;9(1):272.
36. Huang H, Gandhi JK, Zhong X, Wei Y, Gong J, Duh EJ, et al. TNFα is required for late BRB breakdown in diabetic retinopathy, and its inhibition prevents leukostasis and protects vessels and neurons from apoptosis. Invest Ophthalmol Vis Sci. 2011;52(3):1336-44.
37. Liang Z, Gao KP, Wang YX, Liu ZC, Tian L, Yang XZ, et al. RNA sequencing identified specific circulating miRNA biomarkers for early detection of diabetes retinopathy. American Journal of Physiology-Endocrinology and Metabolism. 2018;315(3):E374-E85.
38. Khan R, Kadamkode V, Kesharwani D, Purkayastha S, Banerjee G, Datta M. Circulatory miR-98-5p levels are deregulated during diabetes and it inhibits proliferation and promotes apoptosis by targeting PPP1R15B in keratinocytes. RNA Biol. 2020;17(2):188-201.

Footnotes and Financial Disclosures

Conflict of interest

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