Network-based inference of master regulators in epithelial membrane protein 2-treated human RPE cells

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Abstract

Background: The application of cell-specific construction of transcription regulatory networks (TRNs) to identify their master regulators (MRs) in EMP2 induced vascular proliferation disorders has been largely unexplored.

Methods: Different expression gene (DEGs) analyses was processed with DESeq2 R package, for public RNA-seq transcriptome data of EMP2-treated hRPECs versus vector control (VC) or wild type (WT) hRPECs. Virtual Inference of protein activity by Enriched Regulon analysis (VIPER) was used for inferring regulator activity and ARACNE algorithm was conducted to construct TRNs and identify some MRs with DEGs from comparisons.

Results: Functional analysis of DEGs and the module analysis of TRNs demonstrated that over-expressed EMP2 leads to a significant induction in the activity of regulators next to transcription factors and other genes implicated in vascular development, cell proliferation, and protein kinase B signaling, whereas regulators near several genes of platelet activation vascular proliferation were repressed. Among these, PDGFA, ALDH1L2, B4RAP3, ANGPT1 and ST3GAL5 were found differentially expressed and significantly activitve in EMP2-over-expressed hRPECs versus vector control under hypoxia and may thus identified as MRs for EMP2-induced lesion under hypoxia.

Conclusions: MRs obtained in this study might serve as potential biomarkers for EMP2 induced lesion under hypoxia, illustrating gene expression landscapes which might be specific for diabetic retinopathy and might provide improved understanding of the disease.

Keywords: Differentially expressed genes (DEGs), Epithelial membrane protein 2 (EMP2), Retinal pigment epithelial cells (hRPECs), Computational inference, Gene expression, Master regulators (MRs), Transcription regulatory networks (TRNs), Systems biology

Background

Pathologic retinal neovascularization [1] is a potentially blinding consequence seen in many common diseases including diabetic retinopathy [2], retinopathy of prematurity [3], retinal vascular occlusive diseases [4], and neovascular age-related macular degeneration (AMD) [5–7] among others. Epithelial membrane protein 2 (EMP2) has been shown to reasonably modulate activity through neovascularization network in the retinal pigment epithelial cell line ARPE-19 [8, 9] which is reported to be important factors in progress of DR. To date, several studies have applied this approach to compare transcriptomes in vascular proliferation process within various cell types in humans [10, 11], mice [12]. However, to the best of our knowledge, there has been no genome-wide gene expression profiling study specifically comparing the transcriptom within EMP2-treated hRPECs.
Gene expression profiling by RNA sequencing (RNA-Seq) provides an opportunity to better understand the underlying mechanisms of vascular proliferation. Therefore, given the large number of genes potentially involved, it has been challenging to identify which are the core set of genes that play major roles in the pathways or networks. It becomes even more challenging if the recent hypothesis of omnigenic model is true for DR. Thus, it is imperative to identify disease-relevant core gene networks, and possibly the network master regulators (MRs), which, if exist, are more likely to be targeted for therapeutic interventions [13, 14].

ARACNe is an unbiased algorithm that infers direct transcriptional interactions based on the mutual information between each transcriptional regulator and its potential targets [16–18]. Transcriptional regulatory networks (TRNs) conducted by ARACNe summarize the connections between transcription factors and the genes that they regulate, known as their “target genes” [19], in which case, almost all of the genes expressed in a disease-relevant cell type may confer risk for the disease through widespread network interactions with a core set of master regulators (MRs) [9]. With the constructed regulatory networks, MRs could be identified by computing the activity of each regulator (i.e., TFs, TGs, and DEGs) with the R package of VIPER (GitHub commit 0170c27).

Hence, in this study we compared EMP2-treated hRPECs and health control hRPECs at the transcriptome level using RNA-Seq analysis and core gene networks construction technology to identify the determinat MRs as well as the potential underlying pathway which regulating vascular proliferation in hRPECs (Figure S1).

**Results**

All samples had high-quality RNA-seq reads with an average of 96% reads per sample. Reads with mapping quality score (MAPQ)< 10 or those with >5 mismatches in 100 bp aligned region were discarded (Fig. 1A-B).

**Differential Expression of DEGs and functional enrichment analysis of EMP2-treated comparisons**

After low-level processing of 24 EMP2-treated samples and control samples (Fig. 1A-B), we performed differential expression analysis for each treated group comparing with control samples. According to the PCA plot (Fig. 1A), we compared EMP2-OE hRPECs with VC and EMP2-KD hRPECs with WT group. Totally we identified 1239 DEGs in all comparisons of EMP2-treated hRPECs versus VC or WT (Table S1). Figure 2A and B were Venn diagram of DEGs in comparisons under hypoxia and normoxia, respectively (Fig. 2A-B). Figure 2C-D showed up regulated or down regulated DEGs in comparisons under
hypoxia and normoxia, respectively (Fig. 2C-D). Then we performed functional enrichment analysis and found that DEGs of EMP2-OE hRPECs vs VC under hypoxia commonly enriched in positive regulation of cell proliferation, vasculature development, protein kinase B signaling, and negative regulation of platelet activation, and enriched in metabolism and folate biosynthesis pathway (Figure S2A-S2B), which did not show in either EMP2-OE hRPECs vs VC under normoxia, or in EMP2-KD hRPECs vs WT under hypoxia or normoxia (Figs. 3 and 4).

Transcriptome regulatory network (TRN) construction of comparisons of EMP2 treated hRPECs with control

ARACNe was run independently on the four comparisons datasets of DEGs using a conservative mutual information threshold \( p \leq 1.0 \times 10^{-9} \), i.e., \( p \leq 0.05 \) Bonferroni corrected for all candidate interactions. This resulted in highly robust TRNs of transcriptional factors (TFs) and potential target genes (TGs). To further elucidate the regulation and interaction relationship, TRNs were visualized using Cytoscape 3.7.1. In TRNs, a node is either a TG or a TF, an edge is a TF-TF, TF-TG or TG-TG relationship. Totally, 199,093 interactions were counted between 974 TFs and their inferred TGs among four comparisons (Fig. 6A-B, Tables S3-S6).

Regulator activity inference and master regulators (MRs) identification

To further analyze the activity of identified regulators (i.e., DEGs, TFs and TGs), then inferred top MRs, the R package
of VIPER was used to compute the activity of each regulator with the constructed regulatory networks. Of these, 1395 regulators were identified as high activity and their differential expression among four groups under hypoxia were compared by using Venn diagram (Table S2, Fig. 5). In present study, TRNs modules for comparisons of EMP2-treated hRPECs versus VC or WT hRPECs depicted MRs with high intramodular connectivity, i.e., genes most connected with all other genes within the module. Thus, we got PDGFA, ALDH1L2, BA1AP3, ANGPT1 and ST3GAL5 as MRs in the TRN of hypoxia EMP2-OE vs VC hRPECs (Fig. 6A, Table S3), PRSS33 as a MR in the TRN of hypoxia KD vs WT hRPECs (Fig. 6B, Table S4),

**Discussion**

Since early 2010s, RNA-sequencing (RNAseq) has been used to profile transcriptome of retinal neovascularization disease. Differential expression genes (DEGs) analysis can straightforwardly analyze a gene expression dataset. Through DEGs analysis, a set of highly up-regulated and down-regulated genes can be identified by comparing two groups of samples (e.g., hypoxia vs. normoxia). However, gene expression is highly dynamic, and the expression quantification may depend on the techniques (e.g., different platforms of microarray, or RNAseq), making the cross-dataset comparison difficult. The activities of regulators cannot be directly measured by microarray or RNA-seq because these techniques only measure RNA expression level and do not consider gene activity changes by post-translational modifications. Fortunately, activity of regulators can be inferred by its regulons through VIPER, by taking RNA data and a context-specific gene regulatory network (interactome) as inputs [20–22].

In present study, transcriptome analysis of EMP2-overexpressed and knocked down hRPECs under hypoxia or normoxia elucidated eight sets of DEGs which were then used to construct TRNs along with variant analysis. TRNs analyses attempted to simplify the interactions of activity of regulators and emphasized their role in EMP2-treated RPE
A

Fig. 4  KEGG pathway analysis of the DEGs. Bar graph of Kyoto Encyclopedia of Genes, Genomes (KEGG) Pathway analysis. Enrichment of KEGG pathway terms in differentially expressed sequences for EMP2-treated group vs control. The RichR (https://github.com/hurlab/RichR) package was used in enrichment analysis. \( p < 0.01, \) FDR \( q < 0.05, \) overlap cutoff \( > 0.5 \) (A) KEGG Pathway analysis of DEGs under hypoxic condition, (B) KEGG Pathway analysis of DEGs under normoxic condition.
hypoxia, we inferred that when oxidative stress, apoptosis GWAS risk genes [27]. Besides, hypoxia EMP2-altered genes in over-expressed cation [23–26]. Furthermore, we have shown that PDGF barriers by regulating pericyte-endothelial cell communication to the formation of both the blood–brain and blood-retina vascular proliferation, vessel stabilization, and contribute and has been reported to participate in pericyte regulated effects on RPE cells in proliferative retinopathy (PVR), examining the hRPECs under hypoxia. By PDGF) gene network may regulate behavior and food intake by controlling calcium-stimulated exocytosis of neurotransmitters including collagen type III and fibronectin in the pathogenesis of diabetic cataract [17].

Platelet derived growth factor (PDGF) is one of the leading vascular proliferation risk genes identified by GWAS [40]. However, the molecular mechanism underlying the genetic association remains elusive. Here, we have shown computationally that PDGF is one of the top MRs of vascular proliferation factors in hRPECs under hypoxia. By examining the PDGFA-perturbed gene networks in EMP2-over-expressed hRPECs, we found that PDGFA-altered genes in hRPECs are more enriched for gene pathways that are related to proliferation and migration activities. PDGF families were proved to induce the proliferation and migration effects on RPE cells in proliferative retinopathy (PVR), and has been reported to participate in pericyte regulated vascular proliferation, vessel stabilization, and contribute to the formation of both the blood–brain and blood-retina barriers by regulating pericyte-endothelial cell communication [23–26]. Furthermore, we have shown that PDGFA-altered genes in EMP2-over-expressed hRPECs under hypoxia are more enriched for credible inflammation, oxidative stress, apoptosis GWAS risk genes [27]. Besides, we inferred that when EMP2 was over expressed under hypoxia, PDGFA was upregulated and enhanced the interactions with other genes. Thus, multiple lines of evidence from our study suggest that the PDGFA gene network expression activity in the early stages of vascular proliferation may be very important for pathogenesis.

Although our computationally identification of MR has focused on PDGFA, other MRs that we identified here suggest that additional gene networks are relevant to vascular proliferation. The identified MRs contain many of the TFs previously reported in the literature that may potentially be involved in the vascular proliferation pathogenesis of DR, such as ALDH1L2, BAIAAP3, ANGPT1 and ST3GAL5. ALDH1L2 (Aldehyde Dehydrogenase 1 Family Member L2) is a protein coding gene which functions as mitochondrial folate enzyme [28], the maintenance of mitochondrial integrity and energy balance of the cell [29]. The related pathways of LDH1L2 are one carbon pool by folate and metabolism of water-soluble vitamins and cofactors (Figure S2). ALDH1L2 has been previously proven to have correlation with EMP2 in breast cancer cells [30], but it was the first time to be identified in EMP2-treated hRPECs. We also noticed that PDGFA connected with ALDH1L2 by PLSCR4. In this way, PDGFA may mediate accelerated ATP-independent bidirectional trans-bilayer migration of phospholipids upon binding calcium ions, and it will result in a loss of phospholipid asymmetry in the plasma membrane. This process may play a central role in the initiation of fibrin clot formation, in the activation of mast cells as well as in the recognition of apoptotic and injured cells by the reticuloendothelial system. Thus, we thought that over-expression of EMP2 would activate PDGFA through ALDH1L2-PLSCR4 pathway by initializing fibrin clot and recognizing apoptosis.

We also identified that BAIA Associated Protein 3 (BAIAAP3) had correlation with MMP16 [31]. BAIAAP3, functioning in endosome to Golgi retrograde transport, may regulate behavior and food intake by controlling calcium-stimulated exocytosis of neurotransmitters including NPY and serotonin and hormones like insulin. Such neurotransmitters have been proven to have the ability to degrade various components of the extracellular matrix, such as collagen type III and fibronectin in the pathogenesis of diabetic cataract [17].

This investigation was mainly based on ARACNe algorithm analysis of transcriptome information of hRPECs treated by hypoxia and exogenous EMP2. The TRN constructed in this paper provided clues of interaction mechanism between these genes, which not only provided theoretical support for revealing the regulatory relationship among them, but also provided candidate genes, i.e., up-and down-regulated pathway genes, transcriptional factors, and MRs, which could be studied for future SNP discovery work to establish association with different phenotype or traits of interest. We also established a scientific exploration mode for exploring effective targets of
anti-neovascularization, which made the follow-up basic verification and clinical research more reliable. However, the results of bioinformatics analysis ultimately need to be verified by many experiments and combined with clinical practice to carry out gene function and mechanism verification for substantive exploration.

Conclusions
In summary, by using a computational approach, we identified PDGFA, ALDH1L2, BAIAP3, ANGPT1 and ST3GAL5 as MRs that likely contributed to EMP2-induced vascular proliferation in hRPECs under hypoxia. Although powerful, we acknowledged the limitation of
our approach in identifying top MRs, because selecting a top MR was not purely “data-driven”; for instance, PDGFA, ALDH1L2, BAIAP3, ANGPT1 and ST3GAL5 were identified as top MRs among many other MRs was not only data-driven but also based on prior knowledge about their association with vascular proliferation. Nonetheless, our study suggested that MRs in vascular proliferation in EMP2 and hypoxia treated hRPECs could be identified by transcriptional network construction and that MRs such as PDGFA as well as other MRs might constitute convergent gene networks that confer disease risk in a spatial and temporal manner. Therefore, PDGFA and other identified MRs might be a building part of vascular proliferation architecture, which collectively drove DR onset and progression. Transcriptional network construction in larger and more DR-relevant cell types/stages, combined with empirical network perturbation, would further pave the way to deepen our understanding of the genetic contribution to the complex biology of vascular proliferation during DR disease biology.

Methods

Data availability
The RNA-seq data profiles were obtained from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/) GSE151610. The dataset contained 24 samples, which contained mRNA profiles of hRPECs panel consisting of EMP2-knocked-down (KD) hRPECs, EMP2-over-expressed (OE) hRPECs, vector control (VC) hRPECs, and wild type (WT) hRPECs under hypoxia or normoxia. This project was approved by the Institutional Review Board of Sir Run Run Hospital, Nanjing Medical University. All procedures of this study complied with the protocol.

Identification of DEGs
The data set is available at the NCBI Short-Read Archive (SRA), under accession SRA265560. The RNA-Seq data set was mapped in the Homo sapiens (human) genome assembly GRCh38 (hg38) by Hisat2. Feature Counts was used in read counts. After filtering all 0 expression genes, DESeq2 R package was used in different expression gene counting. Cutoff value was absolute log2FC > 1 and P value < 0.05.

GO and KEGG pathway enrichment analysis
We performed functional enrichment analysis using Kyoto Encyclopedia of Genes, Genomes (KEGG) pathway and Gene Ontology (GO) with a p value < 0.05 as the significance cutoff. The RichR (https://github.com/hurlab/RichR) package was used in enrichment analysis (Figure S1).

Transcription regulatory network construction
Regulatory networks were constructed by ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks) [32–34] using significant DEGs. First, mutual interaction between a candidate TF(x) and its potential target (y) was computed by pairwise MI, MI(x, y), using a Gaussian kernel estimator. A threshold was applied on MI based on the null hypothesis of statistical independence (P < 0.05, Bonferroni-corrected for the number of tested pairs). Secondly, the constructed network was trimmed by removing indirect interactions using the data processing inequality (DPI), a property of the MI. Parameters was set to zero DPI (Data Processing Inequality) tolerance and MI (Mutual Information) p-value threshold of 10−8. Therefore, for each (x, y) pair, a path through another TF(z) was considered, and every path pertaining to the following constraint was removed: MI (x, y) < min (MI (x, z),MI (z, y)). A p value threshold of 1 × 10−8 using DPI = 0.1 (as recommended [35]) was used when running ARACNe. Cytoscape software (version: 3.8.0) was used for visualizing the regulatory networks.

Regulator activity inference
The VIPER algorithm had proven to be a useful tool for estimating regulator activity from gene expression data using enriched regulon analysis. With the constructed regulatory networks, we computed the activity of each regulator (i.e., TFs, TGs, and DEGs) using the R package of VIPER (GitHub commit 0170c27). The output of VIPER is a list of highly active MRs as well as their activity scores and their enrichment P values. A set of regulators with significant activity difference were defined at adjusted p-value < 0.01.

Statistical test
All statistical tests and plotting were performed in R 3.5.2 (https://www.r-project.org/) unless otherwise mentioned.

Abbreviations
MRs: Master regulators; EMP2: Epithelial membrane protein 2; hRPECs: Human retinal pigment epithelial cells; TFs: Transcription factors; TGs: Target genes; DEGs: Differential expression genes; VC: Victor control; WT: Wild type; ARACNe: Algorithm for the Reconstruction of Accurate Cellular Networks; VIPER: Virtual Inference of Protein activity by Enriched Regulon analysis; TRNs: Transcription regulatory networks; AMD: Age-related macular degeneration; OE: Over-expressed; KD: Knocked-down; SRA: Short-Read Archive; KEGG: Kyoto Encyclopedia of Genes, Genomes; GO: Gene Ontology.
Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12863-022-01047-9.

Additional file 1. Additional file 2.

Acknowledgements
The authors acknowledge all the members of their respective teams, from Sir Run Run Hospital, Nanjing Medical University, as well as their collaborators (fundamentalist and clinicians), for their support and interaction during many years of involvement with genetics. We thank the developers of the ARACNe and VIPER software for comments on the software usage. We also thank the KEGG copyright permission for these images cited by us and obtained from KEGG in https://www.kegg.jp/kegg/kegg1.html.

Authors’ contributions
Author HW, FC and WG have given substantial contributions to the conception or the design of the manuscript, author WZ and author ZT to acquisition, analysis and interpretation of the data. All authors have participated to drafting the manuscript, author JQ and XL revised it critically. All authors read and approved the final version of the manuscript.

Funding
This work was supported by grants from the National Key Research and Development Plan of China (No. 2020YFC2008505 to Xiang Lu), the National Natural Science Foundation of China (No. 81970218 to Xiang Lu and No. 81970217 to Wei Gao), the Six Talent Peaks Project of Jiangsu Province (No. LGY2019093 to Wei Gao), the Jiangsu Provincial Health Science and Technology Commission (No. WSN-175 to Xiang Lu), Project of Jiangsu Provincial Health Science and Technology Commission (NO.2019093 to Jian Qin) and the Jiangsu Government Scholarship for Overseas Studies (JS-2018–082 to Hua Wan).

Availability of data and materials
The datasets analyzed during the current study are available in the NCBI GEO (https://www.ncbi.nlm.nih.gov/geo/)

Competing interests
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Received: 15 October 2021 Accepted: 17 March 2022 Published online: 07 July 2022

References
1. Plastino F, Pesce NA, André H. MicroRNAs and the HIF/VEGF axis in oculovascular neovascular diseases. Acta Ophthalmol. 2021;99(8):e1255–62. https://doi.org/10.1111/aos.14945.
2. Yu G, Aaberg MT, Patel TP, Jengar RS, Powell C, Tran A, et al. Quantification of Retinal Nonperfusion and Neovascularization With Ultrawidefield Fluorescein Angiography in Patients With Diabetes and Associated Characteristics of Advanced Disease. JAMA Ophthalmol. 2020;138:680.
3. Aouiss A, Anika Idrissi D, Kabine M, Zaid Y. Update of inflammatory proliferative retinopathy: Ischemia, hypoxia and angiogenesis. Current Research in Translational Medicine. 2019;67:62–71.
4. Esmailli DO, Boyer DS. Recent advances in understanding and managing retinal vein occlusions. F1000Res. 2018;7:467.
5. Kiss S, Malangone-Monaco E, Wilson K, Varker H, Stetsovsky D, Smith D, et al. Real-World Injection Frequency and Cost of Ranibizumab and Afiblertcept for the Treatment of Neovascular Age-Related Macular Degeneration and Diabetic Macular Edema. JMCP. 2020;26:253–66.
6. Campochiaro PA, Akhlaq A. Sustained suppression of VEGF for treatment of retinal/choroidal vascular diseases. Prog Retin Eye Res. 2020;83:100921.
7. Ngo Ntjam N, Thulliez M, Paintaud G, Salvo F, Angoulvant D, Pisella P-J, et al. Cardiovascular Adverse Events With Intravitreal Anti-Vascular Endothelial Growth Factor Drugs: A Systematic Review and Meta-analysis of Randomized Clinical Trials. JAMA Ophthalmol. 2021. https://doi.org/10.1001/jamaophthalmol.2021.0680.
8. Sun M, Wadehra M, Casenave C, Lin M-C, Aguirre B, Pankh S, et al. Epithelial Membrane Protein 2 (EMP2) Promotes VEGF-Induced Pathological Neovascularization in Murine Oxygen-Induced Retinopathy. Invest Ophthalmol Vis Sci. 2020;61:3.
9. Telande DG, Yu AK, Forward KM, Morales SA, Morse LS, Park SS, et al. Epithelial Membrane Protein-2 in Human Proliferative Viteoretinopathy and Epiretinal Membranes. Invest Ophthalmol Vis Sci. 2016;57:3112.
10. Liu J, Dakou E, Meng Y, Levys L. Loss of Emp2 compromises cardiogenic differentiation in mouse embryonic stem cells. Biochem Biophys Res Commun. 2019;511:173–8.
11. Yan Y, Jiang R, Yuan P, Wen L, Pang X, Jing Z, et al. Implication of proliferation gene biomarkers in pulmonary hypertension. Anim Models and Exp Med. 2021;4:369–80.
12. Villacampa P, Liyanage SE, Klasa P, Cristante E, Menger KE, Sampson RD, et al. Stabilization of myeloid-derived Hif’s promotes vascular regeneration in retinal ischemia. Angiogenesis. 2020;23:83–90.
13. Corbett BF, You JC, Zhang X, Pyfer MS, Tosi U, Iscione DW, et al. JFoxB Regulates Gene Expression and Cognitive Dysfunction in a Mouse Model of Alzheimer’s Disease. Cell Rep. 2017;20:344–55.
14. Mondragón E, Maher LJ. Anti-Transcriptional Factor RNA Aptamers as Potential Therapeutics. Nucleic Acid Ther. 2016;26:29–43.
15. Singhania A, Graham CM, Gabryelová L, Moreira-Teixeira L, Stavropoulos E, Ptt JM, et al. Transcriptional profiling unveils type I and II interferon networks in blood and tissues across diseases. Nat Commun. 2010;10:2887.
16. Ruggiero AD, Davis A, Sherrill C, Westwood B, Hawkins GA, Palmer ND, et al. Skeletal muscle extracellular matrix remodeling with worsening glycermic control in nonhuman primates. Am J Physiol Regul Integr Comp Physiol. 2021;33:R226–35.
17. Li Y, Jiang S-H, Liu S, Wang Q. Role of IncRNA NEAT1 mediated by YY1 in the development of diabetic cataract via targeting the microRNA-205-3p/ MMP16 axis. Eur Rev Med Pharmacol Sci. 2020;24:5863–70.
18. 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;60:4084.
19. Yang B, Wittkopp PJ. Structure of the Transcriptional Regulatory Network Correlates with Regulatory Divergence in Drosophila. Mol Biol Evol. 2017;34:1352–62.
20. Sánchez-Luis E, Joaquín-García A, Campos-Laborie FJ, Sánchez-Guio F, De Las Rivas J. Deciphering Master Gene Regulators and Associated Networks of Human Mesenchymal Stromal Cells. Biomolecules. 2020;10:557.
21. Langfelder P, Horvath S. WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559.
22. DoostparastTorshizi A, Armoskus C, Zhang H, Forest MP, Zhang S, Souaiaia T, et al. Deconvolution of transcriptional networks identifies TGF-α as a master regulator in schizophrenia. Sci Adv. 2019;5:eaau4139.
23. Alvarez-MI, Shen Y, Giorgi FM, Lachmann A, Ding BB, Ye BH, et al. Functional characterization of somatic mutations in cancer using network-based inference of protein activity. Nat Genet. 2016;48:838–47.
24. Yu B, Chen L, Zhang W, Li Y, Zhang Y, Gao Y, et al. TOP2A and CENPF are synergistic master regulators activated in cervical cancer. BMC Med Genomics. 2020;13:145.
25. Yang Y, Huang X, Ma G, Cui J, Matsubara JA, Kaduassia A, et al. PDGFβR plays an essential role in patient vitreous-stimulated contraction of retinal pigment epithelial cells from epiretinal membranes. Exp Eye Res. 2020;197:108116.
26. Ma J, Tang W, Gu R, Hu F, Zhang L, Wu J, et al. SHP-2-Induced Activation of c-Myc Is Involved in PDGF-B-Regulated Cell Proliferation and Angiogenesis in RMECs. Front Physiol. 2020;11:555006.

27. Gai R, Jiang J. LncRNA ANRIL Silencing Alleviates High Glucose-Induced Inflammation, Oxidative Stress, and Apoptosis via Upregulation of MME in Podocytes. Inflammation. 2020;43:2147–55.

28. Krupenko N, Sharma J, Pediaditakis P, Helke KL, Hall MS, Du X, et al. Aldh112 knockout mouse metabolomics links the loss of the mitochondrial folate enzyme to deregulation of a lipid metabolism observed in rare human disorder. Hum Genomics. 2020;14:41.

29. Sarret C, Ashkavand Z, Paules E, Darboz I, Pediaditakis P, Sumner S, et al. Deleterious mutations in ALDH1L2 suggest a novel cause for neuro-ichthyotic syndrome. NPJ Genom Med. 2019;4:17.

30. Dillard C, Kyohara M, Mah V, McDermott SP, Bazzoun D, Tsui J, et al. EMP2 Is a Novel Regulator of Stemness in Breast Cancer Cells. Mol Cancer Ther. 2020;19:1682–95.

31. Caporarello N, D’Angeli F, Cambria MT, Candido S, Giallongo C, Salmeri M, et al. Pericytes in Microvessels: From “Mural” Function to Brain and Retina Regeneration. JMS. 2019;206351.

32. Hwang S-G, Kim K-H, Lee B-M, Moon J-C. Transcriptome analysis for identifying possible gene regulations during maize root emergence and formation at the initial growth stage. Genes Genom. 2018;40:755–66.

33. Doostparast Torshizi A, Wang K. Deconvolution of Transcriptional Networks in Post-Traumatic Stress Disorder Uncovers Master Regulators Driving Innate Immune System Function. Sci Rep. 2017;7:14486.

34. Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Favera RD, et al. ARACNE: An Algorithm for the Reconstruction of Gene Regulatory Networks in a Mammalian Cellular Context. BMC Bioinformatics. 2006;7:S7.

35. Margolin AA, Wang K, Lim WK, Kustagi M, Nemenman I, Califano A. Reverse engineering cellular networks. Nat Protoc. 2006;1:662–71.

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