Original Article

Construction of Experimentally Validated lncRNA-miRNA-mRNA Regulatory Network in Keratoconus

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Abstract

Background: Keratoconus is a rare genetic eye disease that affects the cornea. It can be passed down from parents to children, and it's hard to know exactly how many people have it. But scientists are working on finding the mechanism of development and pathology of this disease. So, discovering gene expression panels and the regulator factors could modulate Keratoconus treatment. As these regulatory factors, Long non-coding RNAs (LncRNAs) in competition with mRNA can interact with microRNAs (miRNAs), regulating gene expression. However, the roles of competitive endogenous RNA (ceRNA) networks include lncRNAs, miRNAs, and messenger RNAs (mRNAs) remain unclear in Keratoconus. This study was performed to explore novel regulatory networks in Keratoconus disorder.

Material and Methods: The mRNA expression profiles were retrieved from the Gene Expression Omnibus (GEO). Candidates differentially expressed genes (DEGs) were identified to investigate miRNA and mRNA, respectively and construct a lncRNA-miRNA-mRNA network through a comprehensive bioinformatics strategy and analysis.

Results: Through analyzing GSE112155 datasets, 1lncRNA, XIST, was identified. 488 miRNAs and twenty-nine mRNA were obtained as XIST targets from databases that deposit experimental data. So, lncRNA-miRNA- mRNA regulatory network was constructed based on the interactions. Through centralities analysis of the network, top 10 hub nodes (CDKN1A, XIAP, MAPK1, XIST, SP1, AR, LARP1, MACC1, PTEN, EGFR) were discovered. Our data showed that among miRNAs, has-mir-2110 plays key connectivity role in this network. Moreover, by preformed pathway analysis, TGF-beta Signaling Pathway was identified.

Conclusion: Our study provides a novel perspective on the regulatory mechanism of Keratoconus involving Competing for endogenous RNA (ceRNA), including lncRNA, miRNAs, and mRNA.

Keywords: Keratoconus; Competing Endogenous RNA (ceRNA); Differentially Expressed Genes (DEGs); Biomarker; Pathway Analysi.

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Introduction

Keratoconus (KCN) is an eye disease that affects the cornea. It is a relatively common disease with a frequency range from 0.3 to 2,300 per 100,000 with the effect both genders and diverse populations around the world [1] with higher prevalence among Asians and Middle Easters [2]. It has been known that both genetic and environmental factors contribute to the disease occurrence [3, 4]

It's not easy to diagnose Keratoconus because its symptoms can be mistaken for other conditions. However, the disease is characterized by corneal thinning that causes vision problems. It often causes asymmetric astigmatism, meaning one eye is more distorted than the other. However, recent research has uncovered an exciting discovery and introduce many candidate genes such as collagen genes, KC6 and AQP5 could used as hallmark genes for monitoring this conditions and and molecular defect in KC [5-7].

Over the last decades, many studies indicated genetic variation could be correlated with KC, and many mutations were reported [8]. These studies indicated that gene expression status could play an important role in the progression of the disease. It is reported that the expression of VSX1 has been detected in adult corneas during corneal wound healing [9], and downregulation of TGFBI gene, which affects TGF-β pathway, could influence the corneal organization in the KC cornea [10].

Early diagnosis and appropriate treatment are essential for optimal rehabilitation. Histological examination shows that changes are predominantly seen in the epithelium, Bowman's layer, and stroma in KCN corneas. Whether the initial changes occur first in the epithelium or stroma remains unclear and detects high accuracy and specificity biomarker. With the development of sequencing high-throughput techniques, many biological data are produced every day. Among them, gene expression or epigenomics expression profiling could be a good candidate as a biomarker to show cell behavior [11-18].

Understanding gene expression patterns in KCN is essential to investigate the specific pathways that may affect the disease. Recently, several studies showed that many lncRNAs could compete with mRNAs to bind to miRNAs and act as potential ceRNAs to contribute to disease development [19-22]. In this study, the potential competing endogenous RNA (ceRNA) network among lncRNA-miRNA- mRNA has designed. Then, pathway and GO analysis were used for functional analysis of the targeted mRNA that consisted of biological process (BP), cellular component (CC), and molecular function (MF) to clarify the impact of XIST-miRNA-mRNA construction in keratoconus development [23, 24].

Material and Methods

Data Retrieval and Quality Control

The mRNA expression datasets were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). The inclusion criteria were as follows: [1] the type of mRNA expression profiles was RNA-seq; [2] the platform in each dataset were identical; [3] The samples consisted of Keratoconus and Control tissue; [4] The number of samples was reasonable; [5] The samples obtained from human tissue. Finally, the datasets with GSE112155 Accession numbers were selected for analysis in this study.

Data Normalization

The supplementary experimental data were downloaded from GEO. The EdgeR package was applied to data with the Trimmed Mean

of the M-values (TMM) method to normalized data [25, 26].

Identification DEGs

After normalization, to obtain the series matrix file of differentially expressed genes, the Exact test method was used through the EdgeR package to collect and export the expression matrix based on follows criteria to the Retrieval DEGs list: [1] Adj-Pavlue <0.05 [2] |log2(fold-change)| > 2.

To convert gene ID from Ensemble format to official gene symbol, Syngo https://syngoportal.org online web tools to perform the conversion.

Investigation experimental validated XIST target genes and miRNA

To investigate experimental validation XIST targets, Ensemble gene ID converted import to LncRNA2Target v3.0 database (http://bio-annotation.cn/lncrna2target/) [27] and used official symbol in LncTarD database (http://bio-bigdata.hrbmu.edu.cn/LncTarD/) [19]. All experimentally validated targets include 29 genes and 23 mir were extracted.

Investigation experimental validated miRNAs target genes

To explore the miRNAs that Target candidate genes in part 2.4, official gene symbols were imported to mirwalk (http://mirwalk.umm.uni-heidelberg.de/) database [19], and mine the miRNAs approved by mirtarbase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php) validated all data experimentally.

Construction of ceRNA network

The integrated network consists of candidate lncRNA, and potential target mRNAs were constructed. So Cytoscape software [28], version 3.9.1, was utilized to visualize the lncRNA-miRNA- mRNA network.

Gene ontology (GO) AND Pathway analysis of DEGs

GO analysis was used for the identification of enriched GO terms consisted of Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Wikipathway analysis revealed signaling pathway information for these genes. So, to obtain this information, EnrichR online database [20] was analyzed in the GO and KEGG pathway methods.

Construction of Protein-Protein Interaction (PPI) network

We decided to design a PPI network that directly indicated protein interactions to discover interactions among candidate genes. So, used the STRING online database (https://string-db.org/) and tune options to import only experimental reports interactions. All experimental interactions between target mRNA were detected, and the network matrix was exported for visualizing in Cytoscape software. So, the CytoHubba [20] plugin was performed to analyze the network centrality based on the MCC algorithm to identify Hub genes.

Results

Identification of DEGs

The GSE112155 dataset was included in the present study according to the mentioned criteria. This dataset included 10 male keratoconus samples, and in the control group, 4 samples from female and 6 male samples were retrieved. The platform of this dataset was GPL18573 Illumina NextSeq 500, which included six Keratoconus tissue and six

myopia control patients. After Normalization and further quality control analyses (Supplimentory box plot and Rplot), by used EdgeR package based on matrix creation roles and exact test method DEG file was exported [22]. Exported DEGs list was summarized in Supplementary File 1.

After normalization and further analyses by a limma R package, one down regulate RNA statistically Significant was discovered with |log2 (fold-change) | > 2 and P value < 0.05.

Candidate miRNAs

Our included results consist of 2 types of miRNA. The first type is that the mirs are directly targeted by XIST and detected in LncRNA2Target and LncTarD database. In the second type are the mirs that target our candidate mRNAs. The first type targets XIST directly, and the second type could indirectly affect gene expression. So, importing these data together in networks helps find the specific target for our nodes. So, 23 miRNAs experimentally (Table.1) discovered directly interact with XIST and 465 (Supplementary File 2) miRNAs that experimentally validated have interaction with 29 of our candidate genes (Table 2).

Among miRNAs, hsa-mir-17-5p whit 4 connection with another node was discovered as a high degree mir in our network. However, based on the MCC algorithm for hub detection, has-mir-2110 was detected as hub nodes among miRNAs. (Supplementary File 3)

Construction of PPI regulation network and identification of hub-genes

The STRING database provided a PPI regulation network of DEGs. As include criteria, data that were only reported in experimental validation to reveal potential relationships between 29 genes comprehensively was imported. After removing unconnected genes, the remaining target genes were used to establish the PPI

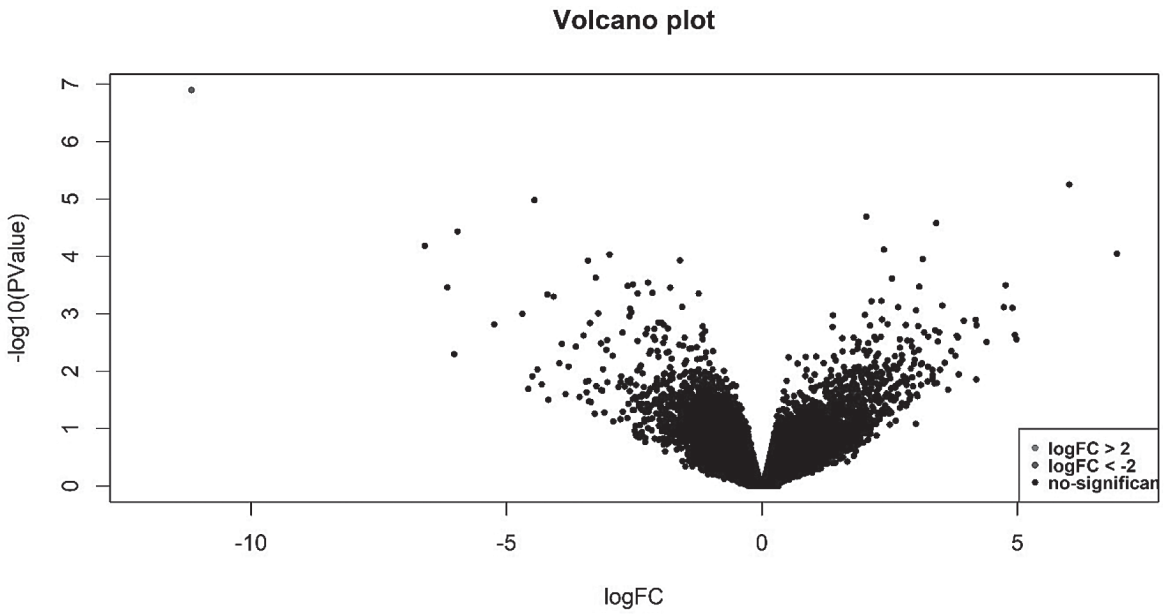


Figure 1: Volcano map for DEGs in Keratoconus. Red spots represent upregulated genes, and blue spots represent downregulated genes. Black spots represent genes without statistically significant change (|log2(fold change) | > 0 and P value < 0.05, to search more statistically significant candidates). As the figure represents, the only XIST sample was altered significantly

Table 1: Experimental XIST in competitive with 23 miRNA validations Details

Target miRNA	lncRNA	Experimental validation method	Study PubMed ID	Disease
hsa-mir-214-3p	XIST	qRT-PCR	30207107	Epithelial ovarian cancer
hsa-mir-152	XIST	qRT-PCR	25578780	Glioblastoma
hsa-mir-155-5p	XIST	qRT-PCR	30091314	Hepatocellular carcinoma
hsa-mir-29c	XIST	qRT-PCR	28831025	Malignant glioma
hsa-mir-101	XIST	qRT-PCR	27620004	Gastric cancer
hsa-mir-137	XIST	qRT-PCR	28287613	Glioma
hsa-mir-320B1	XIST	qRT-PCR	28409547	Osteosarcoma
hsa-mir-140	XIST	qRT-PCR	28656261	Lung cancer
hsa-mir-132	XIST	qRT-PCR	28730777	Colorectal cancer
hsa-mir-let7i	XIST	qRT-PCR	28961027	Lung adeno carcinoma
hsa-mir-21	XIST	qRT-PCR	29048648	Osteosarcoma
hsa-mir-17	XIST	qRT-PCR	29130102	Non- small lung cancer
hsa-mir-138-1	XIST	qRT-PCR	29212249	Bladder Cancer
hsa-mir-130A	XIST	qRT-PCR	29226319	-
hsa-mir-367	XIST	qRT-PCR	29339211	Non- small lung cancer
hsa-mir-195	XIST	qRT-PCR	29384226	Osteosarcoma
hsa-mir-155	XIST	qRT-PCR	29550489	Breast cancer
hsa-mir-124	XIST	qRT-PCR	30114638	Retinoblastoma
hsa-mir-34A	XIST	qRT-PCR	30463570	Thyroid cancer
hsa-mir-494	XIST	qRT-PCR	30551480	Esophageal carcinoma
hsa-mir-133a	XIST	qRT-PCR	31602223	Bladder cancer
hsa-mir-144	XIST	qRT-PCR	31894287	laryngeal squamous cell carcinoma
hsa-mir-454	XIST	qRT-PCR	32098924	triple-negative breast cancer

network. The Network matrix was downloaded to integrate further ceRNA Network analysis.

Construction and analysis of ceRNA network

The current ceRNA network includes four types of connections: mRNA- mRNA, miRNA-mRNA, lncRNA- mRNA, and lncRNA-miRNA (Figure 2). Present ceRNA network consists of 518 nodes and 665 that one node, 448 nodes, and 29 nodes are lncRNA, miRNA, and mRNA, respectively. Through the node ranking analysis of network top, 10 nodes are reported which CDK1N introduced as the top rank node among the other nodes to show the impact of this node in this network. Subsequently, XIST, known as the fourth rank as a lncRNA candidate in the network and the miRNA candidate has-mir 2110 reported as

Table 2: 29 Experimental validated XIST target genes were discover

Target Gene	lncRNA	Expression Status	Experimental validation method	Study Pubmed ID	Disease
AR	XIST	UP	qRT-PCR	28869948	Urinary bladder cancer
PTEN	XIST	Down	qRT-PCR	28388883	Hepatocellular carcinoma
CDKN1A	XIST	UP	qRT-PCR	29254174	Osteosarcoma
BCL2	XIST	UP	qRT-PCR	28248928	Non-small cell lung cancer
TGFB1	XIST	UP	qRT-PCR	29053187	Gastric cancer
PDE4D	XIST	UP	qRT-PCR, Microarray	29226319	Myocardial infarction
ZEB1	XIST	UP	qRT-PCR	30472203	Retinoblastoma
ATG7	XIST	UP	qRT-PCR	29130102	Lung adenocarcinoma
PPP1R13L	XIST	UP	qRT-PCR	28656261	Lung cancer
SGK1	XIST	UP	qRT-PCR	30439718	Colorectal cancer
XIAP	XIST	Down	qRT-PCR, Microarray	12492109	Ovarian cancer
ZEB2	XIST	UP	qRT-PCR	28837144	Colorectal cancer
BAG1	XIST	UP	qRT-PCR	28961027	Lung adenocarcinoma
SMAD7	XIST	Down	qRT-PCR	27100897	Hepatocellular carcinoma
PDCD4	XIST	Down	qRT-PCR	29048648	Osteosarcoma
EZH2	XIST	UP	qRT-PCR	29100288, 27620004	Esophagus carcinoma, Gastric cancer
SP1	XIST	UP	qRT-PCR	28831025	Malignant glioma
PDK1	XIST	UP	qRT-PCR	28231734	Hepatocellular carcinoma
NOD2	XIST	UP	qRT-PCR	29902461	Atherosclerosis
EGFR	XIST	UP	qRT-PCR	28295543	Pancreatic cancer
LARP1	XIST	UP	qRT-PCR	29039571	Lung adenocarcinoma
MGMT	XIST	UP	qRT-PCR	28831025	Malignant glioma
MAPK1	XIST	UP	qRT-PCR	28730777	Colorectal cancer
WNT1	XIST	UP	qRT-PCR	29679755	Colon cancer
RAC1	XIST	UP	qRT-PCR	28469789	Malignant glioma
KLF2	XIST	UP	qRT-PCR	27501756	Non-small cell lung cancer
RSF1	XIST	UP	qRT-PCR	28843909	Osteosarcoma
NOTCH1	XIST	UP	qRT-PCR	29812958	Lung adenocarcinoma
MACC1	XIST	UP	qRT-PCR	27911852	Gastric cancer

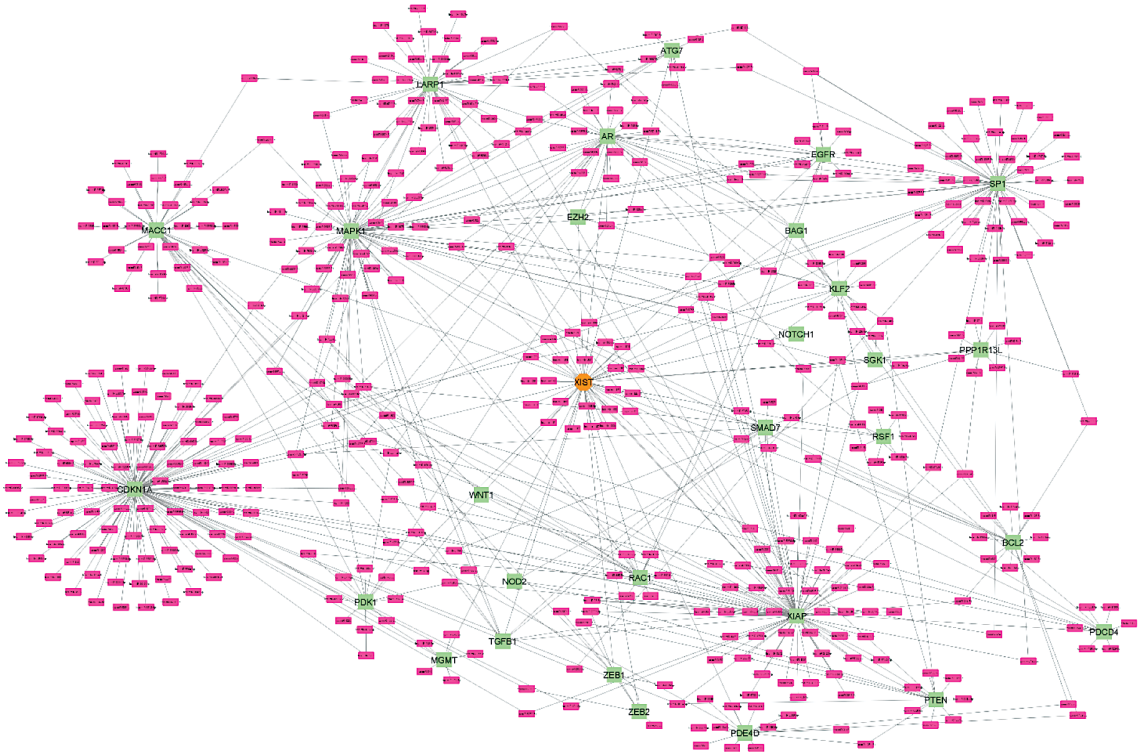


Figure 2: Construction of The ceRNA network consists of 518 nodes and 665 links. The green color nodes present mRNA, The pink nodes present miRNAs, and the orange node presents our candidate lncRNA

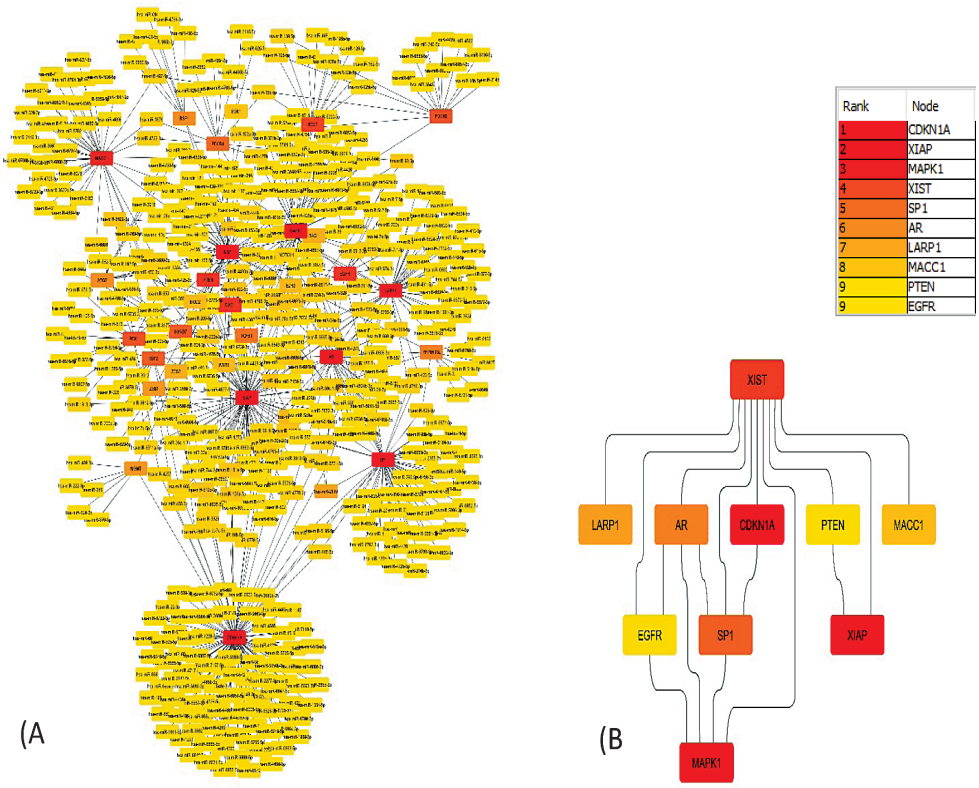


Figure 3: Node ranking analysis. A) Ranking whole network. B) Top 10 hub nodes. The range color from red to yellow represent the highest rank to the lowest based on MCC algorithm

Table 3: Top 10 Pathway analyses of 29 candidate mRNA

Pathway	Adjusted P value	Odds Ratio	Combined Score	Genes
TGF-beta Signaling Pathway WP366	6.63E-11	69.15331	2012.63	*CDKN1A,ZEB2,TGFB1,ZEB1,SP1,MAPK1 ,RAC1,PDK1,SMAD7*
DNA damage response (only ATM dependent) WP710	4.94E-10	70.83066	1870.005	*CDKN1A, TGFB1, PTEN, BCL2, MAPK1, RAC1, WNT1, PDK1*
Integrated breast cancer pathway WP1984	3.98E-09	50.06566	1190.654	*AR, SP1, PTEN, BCL2, MAPK1, RAC1, EGFR, SMAD7*
Androgen receptor signaling pathway WP138	3.98E-09	72.92247	1722.532	*AR, CDKN1A, SP1, BAG1, PTEN, RAC1, EGFR*
Breast cancer pathway WP4262	1.42E-07	41.04141	813.6525	*CDKN1A, NOTCH1, SP1, PTEN, MAPK1, WNT1, EGFR*
Epithelial to mesenchymal transition in colorectal cancer WP4239	1.54E-07	39.42001	770.9928	*ZEB2, NOTCH1, TGFB1, ZEB1, MAPK1, WNT1, EZH2*
Senescence and Autophagy in Cancer WP615	4.01E-07	50.17929	925.796	*CDKN1A, TGFB1, PTEN, BCL2, MAPK1, ATG7*
Chromosomal and microsatellite instability in colorectal cancer WP4216	2.70E-06	58.53529	960.4728	*CDKN1A, TGFB1, BCL2, MAPK1, RAC1*
miRNA regulation of prostate cancer signaling pathways WP3981	5.24E-06	105.7878	1653.256	*AR, CDKN1A, BCL2, MAPK1*
Pancreatic adenocarcinoma pathway WP4263	5.87E-06	47.34762	729.5628	*CDKN1A, TGFB1, MAPK1, RAC1, EGFR*

high connectivity with the network (Figure 3).

GO and Pathway analysis of candidate mRNAs in the network

Because of disease symptoms exposure in phenotype level on mRNA expression, and subsequently translation phase to protein. EnrichR functional analysis online web tools were used to find the role of candidate genes in the development of Keratoconus. So, enriched genes for pathway analysis (Table 3), biological process (BP) (Table 4), cellular component (CC) (Table 5), and molecular function (MF) (Table 6) to clarify the function and pathogenesis in line with clinical evidence. So, results implied that the top pathway-related is TGF-beta Signaling Pathway involved CDKN1A, ZEB2, TGFB1, ZEB1, SP1, MAPK1, RAC1, PDK1, SMAD7 with Adj-P value < 0.05 identified. In BP, positive regulation of cell population proliferation process which AR, CDKN1A, NOTCH1, TGFB1, PTEN, BCL2, NOD2, WNT1, EGFR, EZH2 play an role. Moreover, CC showed

Table 4: Top 10 Biological process (BP) analyses of 29 candidate mRNA

BP	Adjusted P value	Odds Ratio	Combined Score	Genes
positive regulation of cell population proliferation (GO:0008284)	1.17E-06	21.0194	435.6084	*AR,CDKN1A,NOTCH1,TGFB1,PTEN,BCL 2,NOD2,WNT1,EGFR,EZH2*
regulation of cell population proliferation (GO:0042127)	3.40E-06	14.77508	278.277	*AR,CDKN1A,NOTCH1,TGFB1,PTEN,BCL 2,XIAP,WNT1,SGK1,EGFR,EZH2*
negative regulation of cell differentiation (GO:0045596)	3.40E-06	32.72732	599.8333	*NOTCH1,TGFB1,PTEN,PDCD4,WNT1,E GFR,SMAD7*
positive regulation of cellular process (GO:0048522)	3.40E-06	15.73577	284.4347	*AR,NOTCH1,TGFB1,PPP1R13L,PTEN,BC L2,RAC1,WNT1,EGFR,EZH2*
regulation of transcription by RNA polymerase II (GO:0006357)	3.40E-06	9.278539	167.4448	*CDKN1A,NOTCH1,TGFB1,MACC1,PTEN, RSF1,NOD2,EGFR,KLF2,SMAD7,AR,ZEB 2,ZEB1, PPP1R13L,SP1,EZH2*
positive regulation of transcription by RNA polymerase II (GO:0045944)	7.64E-06	12.31022	209.926	*AR,ZEB2,NOTCH1,TGFB1,SP1,MACC1,R SF1,NOD2,EGFR, KLF2,SMAD7*
positive regulation of transcription, DNA-templated (GO:0045893)	9.26E-06	10.70253	178.8116	*AR,ZEB2,NOTCH1,TGFB1,SP1,MACC1,R SF1,NOD2,WNT1, EGFR,KLF2,SMAD7*
regulation of transcription, DNA-templated (GO:0006355)	2.34E-05	7.959175	124.5249	*CDKN1A,NOTCH1,TGFB1,RSF1,EGFR,K LF2,SMAD7,AR,ZEB2,ZEB1,PPP1R13L,S P1,PDCD4,WNT1,EZH2*
regulation of peptidyl-serine phosphorylation (GO:0033135)	4.28E-05	42.74624	638.0181	*TGFB1,PDE4D,PTEN,EGFR,SMAD7*
negative regulation of apoptotic process (GO:0043066)	4.83E-05	14.8603	218.438	*NOTCH1,MGMT,BAG1,BCL2, XIAP,MAPK1,WNT1,EGFR*

that CDKN1A, NOTCH1, TGFB1, MGMT, PDE4D, PTEN, XIAP, RSF1, EGFR, SMAD7, AR, ZEB2, ZEB1, PPP1R13L, SP1, BAG1, BCL2, PDCD4, MAPK1, SGK1, EZH2 exist with high concertation in the nucleus. MF showed that the CDKN1A, BAG1, BCL2, EGFR, SMAD7 genes ubiquitin-protein ligase binding could be noteworthy.

Discussion

Keratoconus is a degenerative eye disease that gradually destroys the cornea tissue. This tissue focuses light onto the retina. Previous studies have indicated that gender and ethnicity

Table 5: 3 significantly Cellular Component (CC) analyses of 29 candidate mRNA

CC	Adjusted P value	Odds Ratio	Combined Score	Genes
nucleus (GO:0005634)	2.88E-06	8.107327	138.74	*CDKN1A,NOTCH1,TGFB1,MGMT,PDE4D,PTEN,XIAP,RSF1,EGFR,SMAD7,AR,ZEB2,ZEB1,PPP1R13L,SP1,BAG1,BCL2,PDCD4,MAPK1,SGK1,EZH2*
intracellular membrane-bounded organelle (GO:0043231)	2.97E-06	7.87234	129.0258	*CDKN1A,NOTCH1,TGFB1,MGMT,PDE4D,PTEN,XIAP,RSF1,EGFR,SMAD7,AR,ZEB2,ZEB1,PPP1R13L,SP1,BAG1,BCL2,PDCD4,MAPK1,RAC1,SGK1,EZH2*
intracellular organelle lumen (GO:0070013)	0.036788	5.679335	37.26029	*TGFB1,MAPK1,WNT1,ATG7,EGFR,PDK1*

could affect KC's morbidity, gene expression, and episodes' age.

Gender consistency in gene expression, however, will generally explain the pathogenesis of KC. In this study, one lncRNA, 488 miRNA, and 29 genes were investigated. Based on our ranking analysis XIST-mir2110-CDKN1A is identified as a key axis of ceRNA networks. In the KC-associated ceRNA regulatory network, there were top 10 hub nodes (including CDKN1A, XIAP, MAPK1, XIST, SP1, AR, LARP1, MACC1, PTEN, EGFR) and has-mir-2110 as high linked miRNA introduced. (Figure3) The candidate mRNA enrichment analysis number of 102 pathways, 504 BP, 3 CC, and 3 MF were significant, adj-pvalue < 0.05, reported.

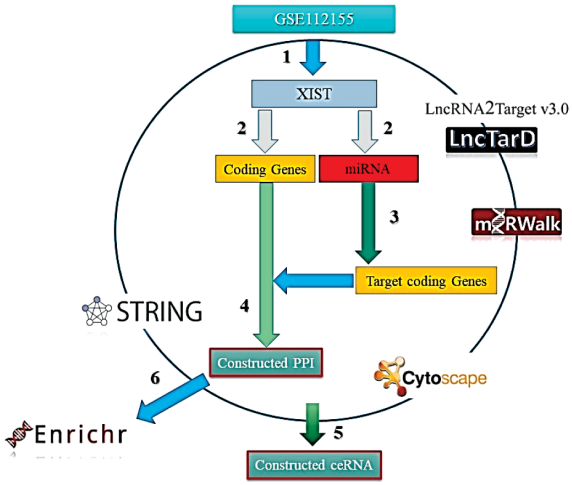
Tiani et al. in 2020 showed that XIST-mir181a-Col4A1 axis might be implicated in the pathogenesis of KC. Our results as the same identify XIST as an eligible candidate based on GSE analysis [29]. However, in contrast to prediction ceRNA nodes, we used experimental validated data and introduced mir2110 and CDKN1A as eligible candidates of miRNA and mRNA respectively in constructed ceRNA networks indicated that based on DEGs analysis, Complement and coagulation cascades is significantly present in Keratoconus.

Karolak et al. in 2016 revealed that VSX1, TGFBI, DOCK9, STK24, IPO5 variations, and

Table 6: 3 significantly Molecular Function (MF) analyses of 29 candidate mRNA

MF	Adjusted P value	Odds Ratio	Combined Score	Genes
ubiquitin protein ligase binding (GO:0031625)	0.003008	15.16154	152.5345	CDKN1A,BAG1,BCL2,EGFR,SMAD7
ubiquitin-like protein ligase binding (GO:0044389)	0.003008	14.21877	138.8493	CDKN1A,BAG1,BCL2,EGFR,SMAD7
type I transforming growth factor-beta receptor binding (GO:0034713)	0.003008	203.7041	1927.344	TGFB1,SMAD7

Work fellow of present suty is available in supplementary figure 1



supplementary figure 1 Workfellow and methodology: 1, Total RNA seq bioproject was selected, and after DEGs analysis, XIST was detected as significant DEGs. 2, Used public databases such as LNcRNA2Target and LncTarD to identify targets. 3, mIRWalk was performed to find miRNA targets. 4, the candidate genes were used to find protein protein interactions. 5, Cytoscape was used to construct the ceRNA network. 6, the included genes in the PPI network were imported to the EnrichR database for further analysis.

DNA damages are involved in the development and progression of KT. In the same way, our results proved that TGFB1 is one of the top 10 key nodes. TGFb pathway signaling and DNA damage response, as top pathways analyzed, is involved in KC progression.

Loukovitis et al., in their review study, summarized the list of up and down-regulation of genes that among them introduced mir-184 as one of the regulation factors which significantly correlated with Keratoconus and clinical symptoms/signs [30]. In contrast, our study, for the first time, indicated that hsa-mir-2110 that targeted AR, SP1, and MAPK1 could be key indirect nodes that regulate major modules of the KC ceRNA network.

Wang YM et al. in 2018 suppose that TaqMan PCR validated the altered expression of four miRNAs in squamous corneal epithelial samples collected from surgery (hsa-miR-151a-3p, hsa-miR-195-5p, hsa-miR-185-5p, and hsa-miR-194-5p) [16, 31-33]. According to the table.1 our study also showed the correlation of hsa-mir-195 with this network before that detected in Osteosarcoma. Given that this mir also could be an eligible candidate to evaluate in bi volume samples.

Conclusion

In summary, throughout the discussion part, we struggled to highlight the importance of attained information through high-throughput technology data analysis and indicate their currently discussed roles in Keratoconus. Our study suggests XIST-mir2110-CDKN1A as main target in ceRNA networks, and 2 major pathways were introduced as potential pathways for further exploration and experimental evaluation.

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Footnotes and Financial Disclosures

Conflict of interest:

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