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Transcriptome Sequencing Reveals the Potential Mechanisms of Modified Electroconvulsive Therapy in Schizophrenia

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Objective Schizophrenia (SCZ) is one of the most common and severe mental disorders. Modified electroconvulsive therapy (MECT) is the most effective therapy for all kinds of SCZ, and the underlying molecular mechanism remains unclear. This study is aim to detect the molecule mechanism by constructing the transcriptome dataset from SCZ patients treated with MECT and health controls (HCs).

Methods Transcriptome sequencing was performed on blood samples of 8 SCZ (BECT: before MECT; AECT: after MECT) and 8 HCs, weighted gene co-expression network analysis (WGCNA) was used to cluster the different expression genes, enrichment and protein-protein interaction (PPI) enrichment analysis were used to detect the related pathways.

Results Three gene modules (black, blue and turquoise) were significantly associated with MECT, enrichment analysis found that the long-term potentiation pathway was associated with MECT. PPI enrichment p-value of black, blue, turquoise module are 0.00127, $<1\times10^{-16}$ and 1.09×10^{-13}, respectively. At the same time, EP300 is a key node in the PPI for genes in black module, which got from the transcriptome sequencing data.

Conclusion It is suggested that the long-term potentiation pathways were associated with biological mechanism of MECT.

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Key Words Electroconvulsive therapy, WGCNA, Network analysis, Schizophrenia.

INTRODUCTION

Schizophrenia (SCZ) is a stressful, chronic, incorrigible psychological disorder,[1] which affecting about 1% of the general population worldwide.[2] Antipsychotics are the mainstay of treatment,[3] but it is associated with important side-effects that can cause serious disability or death.[4] Although multiple treatments are available for SCZ, antipsychotics remain the primary choice.[3] But it was reported that compliance with antipsychotic treatment is affected by many factors.[4]

The effectiveness of electroconvulsive therapy (ECT) in SCZ and mood disorders have been verified.[5] When rapid relief of symptoms is desired, ECT, combined with antipsychotic drugs may be considered an option for patients with SCZ.[6] In contrast to the high response rate of patients with SCZ, little is known about the underlying mechanisms of the action of ECT.

To uncover the mechanisms of the action of ECT, several studies focus on the change of gene expression. Kaneko et al.[7] found that tumor necrosis factor family may play a role in modified electroconvulsive therapy (MECT) by employing microarray analysis of cDNA derived from the peripheral blood of patients with catatonic SCZ. The expression level of cell factors of patients with SCZ changed after MECT was

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also reported.[8] Our study aims to detect the possible genes affected by MECT through analyzing the transcriptome of 8 SCZ (before and after MECT) and 8 HCs.

METHODS

Participants

All the 8 SCZ were recruited in the Fourth People's Hospital of Yibin, PR China. The Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV), Patient Edition (SCID-P)[9] were used to diagnose by the professional trained psychiatrist. All recruited patients meet the enrollment criteria: 1) Age from 18 to 60 years old; 2) The severity of symptom were accessed by the Positive and Negative Syndrome Scale (PANSS)[10] and the total scores of baseline was 60 or more; 3) MECT were not applied during the last 6 months. The exclusion criteria: The severity physical illnesses including neurologic abnormalities, brain injuries and other related diseases to induce mental symptoms, other mental disorder like dementia and fail to understand the content. The 8 HCs were all recruited from the local area by advertisement and accepted the SCID Non-Patient Edition (SCID-NP)[9] to affirm the absence of any mental disorders. All the participants were Han Chinese from the Sichuan province of China. The study was approved by the local Institutional Ethics Committee (IRB number: KY201990). All subjects provided informed consent for participation in the study.

MECT treatment

MECT was applied to all the patients by using the Thymatron IV instrument (Somatics, Lake Bluff, IL, USA). The treatment course of MECT was 6 times, 3 times a week. The patients were evoked using bilateral electrical stimulation with an initial electrical dose that based on 2/3 of their age, and subsequent dosing was performed according to seizure morphology adequacy. EEG can be used to monitor and assess the patient's seizures. The indicators for reference include the peak heart rate, EEG endpoint, average seizure energy index and etc., all the parameters are recommended by instruction of Thymatron. Before treatment, patients received etomidate (0.16−0.2 mg/kg) to reach anesthesia status, succinylcholine (1.0 mg/kg) for muscle-relaxing and atropine sulfate (0.01 mL/kg) to reduce airway secretion by intravenous injection. The entire treatment process is monitored by professional anesthesiologists to prevent serious side effects such as asphyxia and arrhythmia.

Symptom assessment

All the patients received two times PANSS (baseline and end of the MECT treatment) to envaulted the severity of symptoms and the efficiency of the MECT, the PANSS reduction rate was defined as (PANSS score at baseline—PANSS score at the end of ECT treatment)/(PANSS score at baseline—30) ×100%, the reduction rate was indicated as: >75% indicated complete remission, 50−75% significant improvement,[11] so response to ECT was defined as ≥50% PANSS reduction rate and all the patients meet the criterion.

RNA extraction, library preparation, and sequencing

The peripheral blood of the SCZ patients were collected before and after the MECT in anticoagulation tubes and stored in -80°C immediately. The whole blood of the HCs were collected in the same way. All the whole blood samples were used to extract the RNA using the protocol for TRIzol Reagent. The NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and Agilent 2100 (Agilent Technologies, Santa Clara, CA, USA) were used respectively to determine the RNA concentration and integrity number (IN), besides, agarose gel electrophoresis proved the integrity of all the RNA samples, spectrophotometer shows the OD260/280 of all the samples is between 1.8 to 2.0. To construct the RNA-transcriptome library, 5 ug of each high-quality RNA sample was used. The libraries were used for further transcriptome sequencing. In brief, mRNA was isolated according to the poly(A)-oligo(dT) and fragmented by fragmentation buffer, secondly, cDNA was synthesized via random hexamers and Illumina's library construction protocol was used to handle matched cDNA. Libraries were size-selected for cDNA target fragments of 200−300 bp on 2% Low Range Ultra Agarose followed by PCR amplified using Phusion DNA polymerase for 15 PCR cycles. After quantification by TBS-380, a paired-end RNA-seq sequencing library was sequenced with the Illumina HiSeq 4000 system (2×150 bp read length).

Read mapping and the construction of the library

SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) were used to test the quality of raw paired-end reads and detect the default parameters. Then using TopHat (http://tophat.cbcb.umd.edu/, version 2.0.0) 12 software to respectively aligned the clean reads in accordance with the reference genome. The criteria of bowtie mapping were as follows: sequencing reads should be particular matched to the genome with 2 mismatches at most and no insertions or deletions. Under the principle of the whole genome was split into multiple 15 kbp windows that shared 5 kbp. New transcribed regions were defined as more than 2 consecutive windows without overlapped gene regions, where at least 2 reads mapped per window in the same orientation. To identify DEGs between BECT and AECT patients. Expression level of each gene was calculated using the frag-

ments per kilobase of exon per million mapped reads (FRKM) method. RNA Sequencing by Expectation-Maximization (RSEM) (http://deweylab.biostat.wisc.edu/rsem/) was used to define gene abundances.

Construct the WGCNA network

The R package software edgeR[12] was used for differential expression analysis, then the R package WGCNA[13] was used to construct gene co-expression networks based on the different expression genes (DEGs), and the minimum module size was set to 30. Enrichment and PPI analysis were in the supplementary material.

Enrichment analysis

To determine whether the genes in each module were associated with pathophysiological mechanism of SCZ, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Disease Ontology (DO) enrichment analysis of those genes were applied. A hypergeometric test implemented in WebGestalt[14] allowed us to compute the enrichment p-value, which was followed by a Benjamini-Hochberg (BH) correction for multiple testing. The enriched results were reported at a BH-corrected value of $p<0.05$.

Protein–protein interaction analysis

We carried out the permutation test using STRING[15] and evaluated whether genes in the three gene module (Blue, Black and Turquise) had significant physical interactions with each other or with other proteins via the network connectivity parameters (number of edges and degree) versus the random networks with a similar size and degree distribution. Then we utilized the CentiScaPe 2.2[16] tool to identify the key node according to the topological properties of the degree and betweenness in black module .

Gene expression of EP300 in between SCZ and HCs in different brain areas

In order to know whether the expression level of EP300 in different human brain areas have discrepancy, by examining 5 brain regions (cerebellum, parietal cortex brain, hippocampus, prefrontal cortex and striatum) gene expression data of SCZ patients and HCs (all data from http://www.szdb.org/index.html).

Statistical analyses

Multiple analysis was used to test those modules in different group and each module needs to meet the following points: 1) statistical differences for MEs between BECT and HC; 2) statistical differences for MEs between BECT and AECT group; 3) no statistical difference for MEs between AECT and HC group. Chi-square test were used to examine the differences of age, gender, marital status resident areas; t-test was used to examine the differences of educated years of patients and HCs, and PANSS scores (BECT and AECT) also use t-test to check the statistic difference. SPSS version 23 (IBM Corp., Armonk, NY, USA) was used to all the data analysis.

RESULTS

Description of clinical information

The demographic differences and statistic results are all shown in the Table 1. There is no statistical variability between SCZ and HC group in age, gender, educated years, marriage status and urban-rural differences. Among the 8 SCZ patients, before the MECT they all received the antipsychotic medication treatment, in addition to the use of antipsychotic drugs during the MECT, 2 patients used propranolol to control their heart rate, 2 patients used a combination of benzodiazepines to improve sleep quality, and another patient add a mood stabilizer; except one female patient has the positive family history of mental disorder, others are all negative family history. PANSS scores and other clinical information are shown in Table 1.

Construct the WGCNA network

To investigate the expression of genes affected by MECT in SCZ. Firstly, 3000 DEGs ($p<0.05$) were obtained after analyzing the transcriptome sequence of 8 SCZ patients, then those DEGs of the 8 SCZ patients and 8 HCs were used to construct the gene co-expression network through WGCNA and 13 modules were obtained.

Compare the MEs of each module

According to the above-mentioned WGCNA analysis, multiple comparisons were used to compare MEs in each module in SCZ patients (BECT vs. AECT) and HCs, it found that three modules (Black, Blue, and Turquoise) whose MEs were significantly associated with disease and treatment status (Table 2).

Gene sets enrichment analysis

Results of KEGG pathway are following points: One important pathway in black module was found: long-term potentiation (Table 3, Supplementary Table 1 in the online-only Data Supplement). Other pathways also have the close connections to mental, nervous disorders, chromosome aberrations and so on (Supplementary Table 2 in the online-only Data Supplement). Besides, it was found that the black module was also associated with drug valproic acid (Table 3, Supplementary Table 3 in the online-only Data Supplement).

Table 1. Demographic and clinical data

Variables	SCZ (N=8)	HC (N=8)	χ^2/t	p
Age, mean	31.63±6.28	31.25±11.22	t=0.30	0.75
Gender, women (%)	4 (50)	4 (50)	χ^2=0	1
Educated years, N (%)				
Primary	0 (0)	2 (25)	χ^2=3.54	0.17
Middle	5 (62.5)	3 (37.5)	χ^2=2.4	0.30
High & above	3 (37.5)	3 (37.5)	χ^2=0	1.00
Baseline PANSS score	83.63±7.11		t=1.36	0.19
AECT PANSS score	39.88±6.92		t=6.07	<0.01
Medicine, N (%)				
Risperidone (3–6 mg/d)	4 (50)	-		
Sulpiride (0.3–0.6 g/d)	2 (25)	-		
Aripiprazole (10–15 mg/d)	2 (25)	-		
Married (%)	4 (50)	4 (50)		
Urban & rural (%)	6 (75)	6 (75)		

Statistic demographic data results, there is three levels in educated years, primary refer to the 1–6 educated years, middle refer to 7–12 years and high & above refer to 13 or longer educated years. SCZ: schizophrenia, HCs: health controls, BECT: before MECT, AECT: after MECT, PANSS: Positive and Negative Syndrome Scale

PPI network analysis of genes in the three gene modules

The findings indicated that the richness in protein connections in each module and the resulting network was significantly different from any random networks; there were 51 direct edges in the black module gene network compared with only 32 edges expected (p=0.00127) (Supplementary Figure 1A in the online-only Data Supplement), 493 direct edges in the blue module gene network compared with only 309 edges expected (p<0.001) (Supplementary Figure 1B in the online-only Data Supplement), 653 direct edges in the turquoise module network compared with only 483 edges expected (p<0.001) (Supplementary Figure 1C in the online-only Data Supplement). Those results suggest that the networks generated from genes in the three gene modules did not occur by chance. Meanwhile, EP300 is also a core node in the PPI network for black module, which has 10 degrees and 17.3 betweenness (Supplementary Table 4 in the online-only Data Supplement).

Expression of key gene EP300 in different brain areas between SCZ and HCs

By examining the gene expression level of EP300 in the brain regions mentioned above, we identified three areas including hippocampus (p=0.024), prefrontal cortex (p=0.012), cerebellum ($p=1.35\times10^{-4}$). After the false discovery rate (FDR), it found that gene expression level of EP300 in cerebellum was statistic different (FDR=0.026) (Figure 1). Besides, from the results of hub genes in PPI for black module, it found that EP300 was hub gene, which participated in LTP pathway meanwhile.

Table 2. Multiple comparisons of 13 module eigengenes

Module	BECT & AECT	BECT & HCs	AECT & HCs
Tan	0.001	0.277	0.010
Black	0.003	<0.001	0.352
Pink	0.014	<0.001	0.045
Blue	0.006	0.009	0.827
Turquoise	0.026	0.004	0.405
Magenta	0.087	0.159	0.742
Salmon	0.317	0.311	0.991
Green	0.539	0.547	0.991
Yellow	0.537	0.220	0.532
Greenyellow	0.758	0.917	0.680
Purple	0.778	0.432	0.611
Brown	0.754	0.714	0.498
Red	0.959	0.867	0.908

HCs: health controls, AECT: patients before modified electroconvulsive therapy, BECT: patients after modified electroconvulsive therapy

DISCUSSION

It was found that gene expression changes in patients treated with MECT. Nishiguchi et al.[17] found the mRNA expression of Yamanaka's four transcription factors in the peripheral blood showed a tendency toward higher levels after MECT, the mRNA level of synapsin II was significantly upregulated

Table 3. Results of enrichment analysis for genes in the black module

Category	Pathway	ID	P	P_{adjust}	Gene module
KEGG	Long-term potentiation	4720	5.49e-05	0.0016	Black
	Phosphatidylinositol signaling system	4070	0.0004	0.0058	Black
	Regulation of actin cytoskeleton	4810	0.0004	0.0058	Black
Drug	Valproic acid	PA451846	0.0074	0.0222	Black

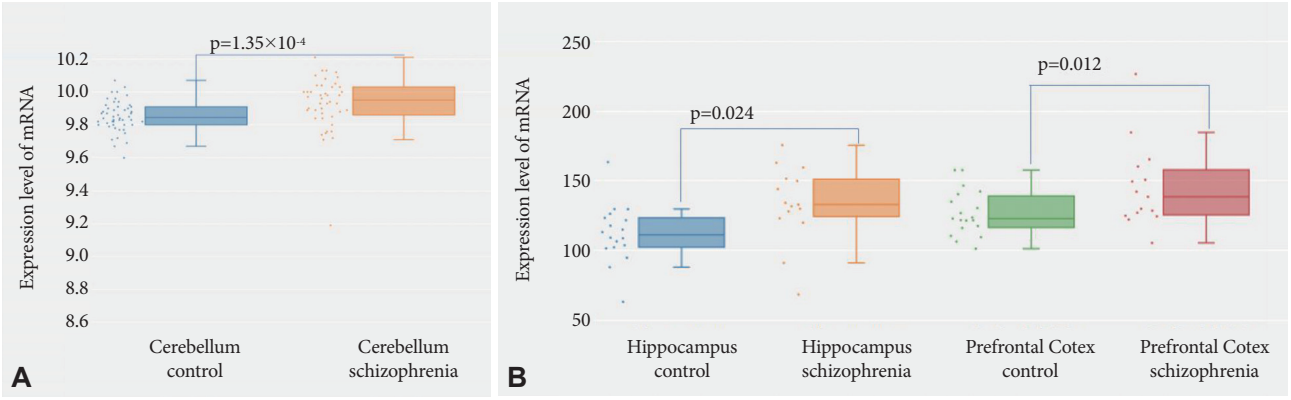

Figure 1. Gene expression level of EP300 in different brain areas. A: The gene expression levels of EP300 in cerebellum areas between patients with SCZ and controls. B: The gene expression levels of EP300 were compared in hippocampus and prefrontal cortex between patients with SCZ and controls. The X-axis indicates the gene expression level of EP300 in different brain regions, and the Y-axis indicates the amount of gene expression.

after repeated ECS.[18] To our knowledge, this is the first study focus on the differences of transcriptomes between SCZ patients treated with MECT and HCs.

Long-term potentiation (LTP) is also an important pathway in black module, it participated in variety of SCZ-related biological pathways. Like long-term plasticity of synaptic transmission, such as in LTP and long-term depression (LTD), provides a cellular correlate of experience-driven learning.[19] As identified by GWAS,[20] shared KEGG pathways related to SCZ including LTP.[20] However, variation in practice of ECT can lead to cognitive side-effects,[21] which memory impairment is the most worrisome, it is associated with both anterograde and retrograde amnesia,[22] although a recently study concluded that there is no evidence of cumulative cognitive deficits associated with repeated ECT courses.[23] Converging lines of evidence suggest that cognitive deficits are associated with impaired LTP. Alterations of dopaminergic, GABAergic, and glutamatergic neurotransmission could all lead to impaired LTP in SCZ,[24] both LTP and LTD represent morphological and functional changes occurring in the process of memory formation.[25] Our results identified this important signal pathways could provide new clue for understanding the genetic architecture of MECT in SCZ.

DO enrichment analysis showed that genes in the black module were related to valproic acid (VPA). VPA was found to induce broader epigenetic changes through different mechanisms, like DNA demethylation and histones acetylation.[26] Genotypic variations can influence methylation levels of the CHRNA7 promoter, which is linked with SCZ, it has been proven that epigenetic dysregulation can be reversed by valproate, which caused demethylation and increased CHRNA7 expression in Hela cells.[27] Combine the found of Paulsen et al.,[28] they revealed the presence of elevated levels of potassium and zinc in SCZ NPCs (neural progenitor cells), neural cells treated with valproate brought potassium and zinc content back to control levels. Besides, an animal study focus on plasticity-related mechanisms in the neocortex of 2-week-old rats prenatally exposed to VPA, it indicate that VPA significantly enhances NMDA receptor-mediated transmission and causes increased plasticity in the neocortex.[29] More importantly, a study shows that treatment with VPA make the enrichment of Ep300 and Kdm6b in PP but not PE of Pou5f1 promoter, which POU5F1 is essential for maintaining pluripotency in embryonic stem cells (ESCs).[30]

In summaries, VPA plays an important role in SCZ and symptoms relief in SCZ may partly rely on MECT mediated VPA pathway alteration.

It has known to all that MECT also could improve the cognitive function. it was indicated that eight genes of some loci were associated with cognitive deficits of SCZ, EP300 is one of it.[31] In addition, EP300 may become the key gene to influence the epigenetic mechanisms, which including gene expression, and synaptic plasticity.[31] A study using a combination of relative enrichment score (RES) and conditional FDR

to elucidate the links between neuroticism and complex diseases, it was found that three SNPs are located in intronic regions of EP300 which have been reported to be associated with autism, Parkinson's disease and SCZ, respectively.[32] Besides, single SNP analyses revealed that rs9607782, located near EP300, was significantly associated with amygdala recruitment during emotion processing.[33] A mutation burden test found gene the strawberry notch homolog 1 (SBNO1), in the exons of this gene found different mutation burden profile from the EP300,[34] which loss of one copy of EP300 leads to abnormal neurodevelopment.[35] From all those findings, it can confirmed that EP300 is not only the key gene in black module, but also participated in so many functions related to SCZ, it can infer that MECT may change the related pathways, and further studies are needed to reveal the underlying mechanism of effects of EP300 on cognitive function, which may expand our understanding of how MECT improves cognitive impairment in SCZ.

In conclusion, our work still has some shortcomings and limitations. The insufficient amount of the samples is a great shortage; the influence of all the antipsychotics should be considered into the assessment of the severity of the symptoms, the impact of mono-antipsychotic, MECT and antipsychotics combined MECT treatment to gene expressions levels should be considered in the future researches, the gene expression changes in different time points in MECT therapy, the gene expression profile of MECT-resistance patients and etc. What is more, the transcriptome data of other SCZ related brain areas should also be detected to compare the DEGs to find more possible pathways. Besides, the pathways that revealed by our transcriptome data play a vital role in multiple neural functions, include cognitive function, neurodevelopment, synaptic plasticity and etc.

From all the above results, it is informed that many previous studies and findings were based on the change of single gene and/or protein of SCZ. Our study identified the long-term potentiation pathway and gene EP300 may play an important role in MECT and provides a new and macro perspectives for the future studies which devoted to understand the possible mechanism of MECT in SCZ treatment.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.30773/pi.2020.0410.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Wanhong Peng, Qingyu Tan, Ping Wang, Bo Xiang, Kezhi Liu, Xuemei Liang. Data curation: Wanhong Peng, Qingyu Tan, Minglan Yu, Ping Wang, Tingting Wang, Jixiang Yuan. Formal analysis: Wanhong Peng, Qingyu Tan, Ping Wang, Bo Xiang. Funding acquisition: Wanhong Peng, Dongmei Liu, Dechao Chen, Chaohua Huang, Youguo Tan, Kezhi Liu, Bo Xiang, Xuemei Liang. Investigation: Wanhong Peng, Qingyu Tan, Minglan Yu, Ping Wang, Tingting Wang, Jixiang Yuan. Methodology: Wanhong Peng, Qingyu Tan, Ping Wang, Bo Xiang. Project administration: Wanhong Peng, Dongmei Liu, Dechao Chen, Chaohua Huang, Youguo Tan, Kezhi Liu, Bo Xiang, Xuemei Liang. Resources: Wanhong Peng, Dongmei Liu, Dechao Chen, Kezhi Liu, Bo Xiang, Xuemei Liang. Validation Software: Wanhong Peng, Ping Wang, Bo Xiang. Supervision: Dongmei Liu, Dechao Chen, Chaohua Huang, Youguo Tan, Kezhi Liu, Bo Xiang, Xuemei Liang. Validation: all authors. Visualization: all authors. Writing—original draft: Wanhong Peng. Writing—review & editing: all authors.

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Supplementary Table 1. The results of KEGG enrichment analysis of genes in three gene modules

KEGG pathway	KEGG ID	P	P_{adjust}	Gene module
Long-term potentiation	4720	5.49e-05	0.0016	Black
Regulation of actin cytoskeleton	4810	0.0004	0.0058	Black
Phosphatidylinositol signaling system	4070	0.0016	0.0155	Black
Leishmaniasis	5140	0.0189	0.0520	Black
Focal adhesion	4510	0.0211	0.0520	Black
Hypertrophic cardiomyopathy (HCM)	5410	0.0247	0.0520	Black
Calcium signaling pathway	4020	0.0153	0.0520	Black
Wnt signaling pathway	4310	0.0098	0.0520	Black
Endometrial cancer	5213	0.0102	0.0520	Black
Prostate cancer	5215	0.0281	0.0520	Black
Osteoclast differentiation	4380	2.18e-12	2.57e-10	Blue
Hepatitis C	5160	4.45e-12	2.63e-10	Blue
Toll-like receptor signaling pathway	4620	1.43e-11	5.62e-10	Blue
Neurotrophin signaling pathway	4722	2.87e-10	8.47e-09	Blue
Pancreatic cancer	5212	5.00e-10	1.13e-08	Blue
Non-small cell lung cancer	5223	5.74e-10	1.13e-08	Blue
Leukocyte transendothelial migration	4670	1.01e-09	1.70e-08	Blue
Fc epsilon RI signaling pathway	4664	1.90e-09	2.80e-08	Blue
T cell receptor signaling pathway	4660	4.93e-09	6.46e-08	Blue
Prostate cancer	5215	6.94e-09	8.19e-08	Blue
Protein processing in endoplasmic reticulum	4141	1.51e-09	1.83e-07	Turquoise
Ubiquitin mediated proteolysis	4120	3.86e-08	1.56e-06	Turquoise
Endocytosis	4144	2.98e-08	1.56e-06	Turquoise
Hepatitis C	5160	2.58e-07;	7.80e-06	Turquoise
Cell cycle	4110	7.62e-07	1.84e-05	Turquoise
Neurotrophin signaling pathway	4722	9.86e-07	1.99e-05	Turquoise
Prostate cancer	5215	1.62e-06	2.31e-05	Turquoise
Pathways in cancer	5200	1.58e-06	2.31e-05	Turquoise
Renal cell carcinoma	5211	1.72e-06	2.31e-05	Turquoise
Regulation of actin cytoskeleton	4810	1.98e-06	2.40e-05	Turquoise

Supplementary Table 2. The results of DO (disease) enrichment analysis of genes in three gene modules

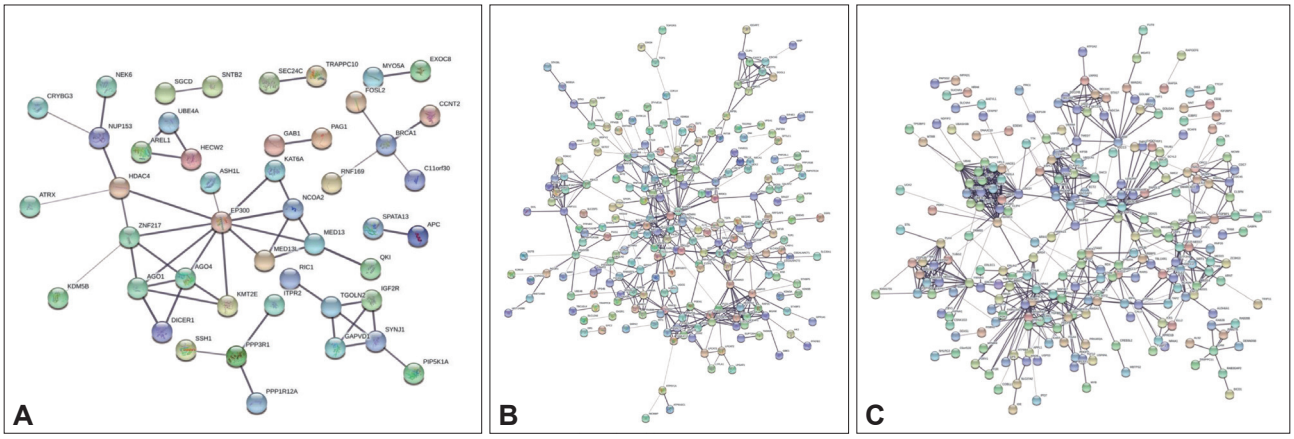
Gene set	ID	P	P_{adjust}	Gene module
Li-Fraumeni syndrome	DB_ID:PA165108952	1.18e-05	0.0018	Black
Translocation, genetic	DB_ID:PA445914	4.19e-05	0.0032	Black
Myotonic dystrophy	DB_ID:PA445031	0.0004	0.0087	Black
Raynaud disease	DB_ID:PA445499	0.0002	0.0087	Black
Mental disorders	DB_ID:PA447208	0.0003	0.0087	Black
Generalised lentiginosis	DB_ID:PA165108848	0.0003	0.0087	Black
Anemia	DB_ID:PA443340	0.0004	0.0087	Black
Nervous system diseases	DB_ID:PA445093	0.0010	0.0191	Black
Leukemia	DB_ID:PA444750	0.0022	0.0262	Black
Muscle hypotonia	DB_ID:PA444992	0.0021	0.0262	Black
Amyotrophic lateral sclerosis	HP:0007354	1.45e-05	0.0158	Blue
Vascular skin abnormality	HP:0007354	0.0011	0.1332	Blue
Atrophy/degeneration involving motor neurons	HP:0007373	0.0006	0.1332	Blue
Abnormality of the dorsal column of the spinal cord	HP:0011397	0.0010	0.1332	Blue
Decreased sensory nerve conduction velocity	HP:0003448	0.0010	0.1332	Blue
Abnormal bleeding	HP:0001892	0.0009	0.1332	Blue
Frontotemporal dementia	HP:0001892	0.0010	0.1332	Blue
Internal hemorrhage	HP:0011029	0.0011	0.1332	Blue
Abnormality of blood circulation	HP:0011028	0.0011	0.1332	Blue
Peripheral neuropathy	HP:0009830	0.0019	0.1713	Blue
cancer or viral infections	DB_ID:PA128407012	4.12e-11	3.09e-08	Turquoise
Neoplasms	DB_ID:PA445062	6.29e-09	2.36e-06	Turquoise
H syndrome	DB_ID:PA162372881	1.25e-07	3.12e-05	Turquoise
Syndrome	DB_ID:PA445789	1.00e-06	0.0002	Turquoise
Pigmentation disorders	DB_ID:PA445325	1.58e-06	0.0002	Turquoise
Mycosis fungoides	DB_ID:PA445010	1.01e-06	0.0002	Turquoise
Neoplasm of unspecified nature of digestive system	DB_ID:PA165108442	3.66e-06	0.0003	Turquoise
Lymphoma	DB_ID:PA444840	3.18e-06	0.0003	Turquoise
Chromosome aberrations	DB_ID:PA443728	2.78e-06;	0.0003	Turquoise

Supplementary Table 3. The results of DO (Drug) enrichment analysis of genes in three gene modules

Drugs	ID	P	Padjust	Gene module
Daunorubicin	DB_ID:PA449212	0.0026	0.0222	Black
Divalproex sodium	DB_ID:PA164783479	0.0074	0.0222	Black
Valproic acid	DB_ID:PA451846	0.0074	0.0222	Black
Cisplatin	DB_ID:PA449014	0.0074	0.0222	Black
Phenylephrine	DB_ID:PA449014	0.0050	0.0222	Black
Doxorubicin	DB_ID:PA449412	0.0035	0.0222	Black
Quinine	DB_ID:PA451213	0.0126	0.0284	Black
Cyclosporine	DB_ID:PA449167	0.0118	0.0284	Black
Glutathione	DB_ID:PA449167	0.0181	0.0358	Black
Tamoxifen	DB_ID:PA451581	0.0199	0.0358	Black
Glutathione	DB_ID:PA449780	1.13e-10	1.89e-08	Blue
Adenosine	DB_ID:PA449780	5.28e-08	4.41e-06	Blue
Dexamethasone	DB_ID:PA449247	9.18e-06	0.0005	Blue
Imatinib	DB_ID:PA10804	1.24e-05	0.0005	Blue
Sirolimus	DB_ID:PA451365	0.0001	0.0024	Blue
Adalimumab	DB_ID:PA10004	8.30e-05	0.0024	Blue
Rituximab	DB_ID:PA451261	0.0001	0.0024	Blue
Progesterone	DB_ID:PA451123	0.0002	0.0037	Blue
Fluocinolone acetonide	DB_ID:PA164754912	0.0002	0.0037	Blue
Urokinase	DB_ID:PA164754912	0.0003	0.0046	Blue
Adenosine	DB_ID:PA448049	3.71e-15	4.97e-13	Turquoise
Adenosine triphosphate	DB_ID:PA164743471	1.87e-06	0.0001	Turquoise
Glutathione	DB_ID:PA449780	4.10e-05	0.0018	Turquoise
Cisplatin	DB_ID:PA449780	0.0003	0.0100	Turquoise
Insulin recombinant	DB_ID:PA164744571	0.0004	0.0107	Turquoise
Sorafenib	DB_ID:PA7000	0.0006	0.0134	Turquoise
Allopurinol	DB_ID:PA448320	0.0011	0.0211	Turquoise
Mitomycin	DB_ID:PA448320	0.0013	0.0218	Turquoise
Ganciclovir	DB_ID:PA449733	0.0018	0.0231	Turquoise
Iodixanol	DB_ID:PA164783998	0.0019	0.0231	Turquoise

Supplementary Table 4. Prioritization of genes in black module

Gene	Betweenness	Degree
EP300	171.3333333	10
HDAC4	110	4
NUP153	62	3
ZNF217	47	5
MED13	32	4
AGO1	16.33333333	5
AGO4	16.33333333	5
BRCA1	12	4
SYNJ1	8	4
TGOLN2	8	4
PPP3R1	6	3
NCOA2	3	4
GAPVD1	0	3
IGF2R	0	3
KMT2E	0	3
MED13L	0	3
AREL1	0	2
DICER1	0	2
HECW2	0	2
KAT6A	0	2
UBE4A	0	2
APC	0	1
ASH1L	0	1
ATRX	0	1
C11orf30	0	1
CCNT2	0	1
CRYBG3	0	1
EXOC8	0	1
FOSL2	0	1
GAB1	0	1
ITPR2	0	1
KDM5B	0	1
MYO5A	0	1
NEK6	0	1
PAG1	0	1
PIP5K1A	0	1
PPP1R12A	0	1
QKI	0	1
RIC1	0	1
RNF169	0	1
SEC24C	0	1
SNTB2	0	1
SPATA13	0	1
SSH1	0	1
TRAPPC10	0	1



Supplementary Figure 1. Results of PPI network analysis of genes in the three gene modules. A: Black module gene. B: Blue module gene. C: Turquoise module gene.